Integration of metabolism

1. **Energetic integration** (NTPs (mainly ATP) are the universal currency of energy; main electron carriers in oxidative reactions are NADH and $FADH_2$, main electron donor in reductive biosynthesis is NADPH)
2. **Metabolic reactions integration in pathways** (few key metabolites, that are key junctions/nods of metabolic pathways; biomolecules are constructed from a small set of building blocks; enzymes' activity is tightly regulated)
3. **Compartmentation of metabolism** (metabolic reactions are compartmented; biosynthesis and catabolism are usually separated in space; compartmentation is supported by regulated molecules' transport, sorting and assembly; specific proteins work for cytoplasm movement, vesicular transport and organelles dynamics)

→ **Results in complex regulation of cell processes (adaptation to environment stresses)**

ГОМЕОСТАЗ. СТРЕСС.

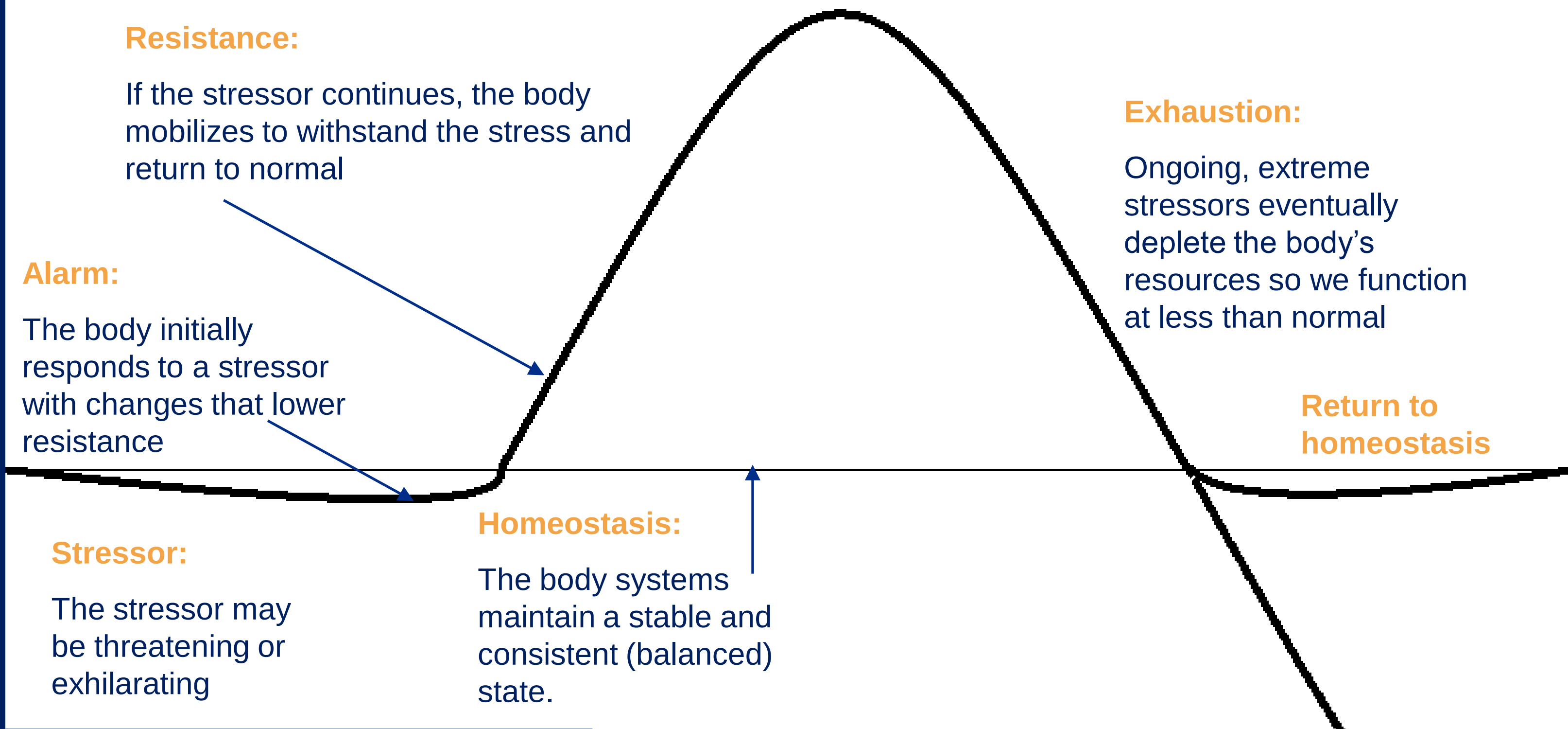
Homeostasis

- Walter Cannon (1929-39)
  - "Homeostasis"
    - Greek *homoios* (similar) and *stasis* (position)
    - The physiological processes that maintain internal stability in an organism.
    - Includes mechanisms for protection against hunger, body temperature, sugar levels, hormone levels, etc.

The Stress Response (Fight or Flight Response)

- Hans Selye (1956)
  - Universal Triad
    - Adrenal glands
    - Thymus glands
    - Gastrointestinal lining
  - General Adaptation Syndrome
    1. Alarm
    2. Resistance
    3. Exhaustion
  - Nonspecific

The three stages of Selye's General Adaptation Syndrome and their consequences



Resistance:

If the stressor continues, the body mobilizes to withstand the stress and return to normal

Alarm:

The body initially responds to a stressor with changes that lower resistance

Stressor:

The stressor may be threatening or exhilarating

Homeostasis:

The body systems maintain a stable and consistent (balanced) state.

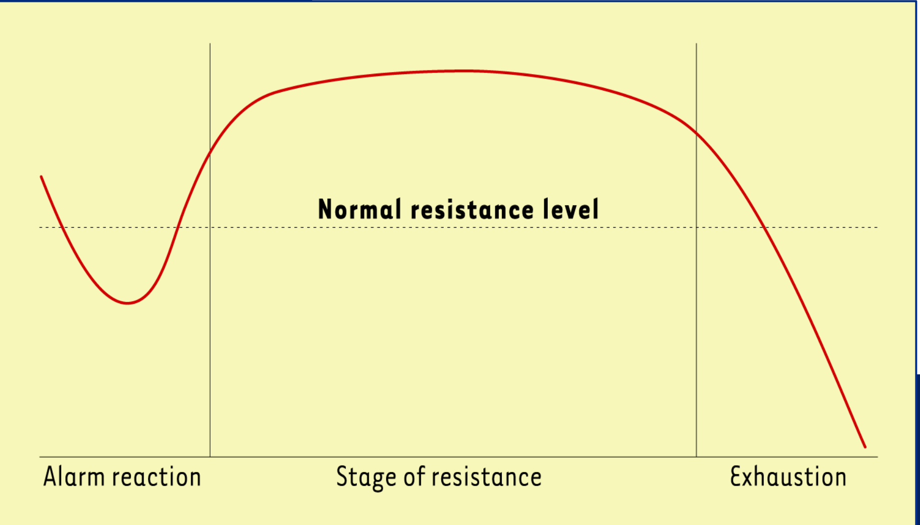
Exhaustion:

Ongoing, extreme stressors eventually deplete the body's resources so we function at less than normal

Return to homeostasis

Illness and death:

The body's resources are not replenished and/or additional stressors occur: the body suffers breakdowns.



Symptoms of Fight or Flight

- Increased heart rate
- Increased blood rate
- Increased ventilation
- Vasodilatation of arteries to body’s periphery (arm and legs)
- Increased serum glucose levels
- Increased free fatty acid mobilization
- Increased blood coagulation and decreased clotting
- Increased muscular strength
- Decreased gastric movement
- Increased perspiration to cool body core temperature

Three Types of Stress

1. Eustress: good stress (e.g., falling in love)
2. Neustress: neutral stress (e.g., earthquake in remote corner of world)
3. Distress: bad stress (e. g., death of a close friend); acute stress (high intensity, short duration); chronic stress* (low intensity, prolonged time)

* Seems to cause the most problems with disease & illness

Agents/treatments that induce the stress response

Conditions	**Probable effects**
temperature shifts (*e.g.*, heat-shock)	protein denaturation
heavy metals, arsenite, iodoacetamide	reaction with sulfhydryl goups, conformational changes in protein
anoxia, hydrogen peroxide, superoxide ions, free radicals	oxygen toxicity, free radical fragmentation of proteins
proteasome inhibitors (lactacystin)	inhibition of proteolysis
amytal, azide, dinitrophenol, ionophores, rotenone, antimycin	inhibition of oxidative phosphorylation, changes in redox state, covalent modification of proteins
hydroxylamine	cleavage of asparagine-glycine bonds
ethanol	translation errors
amino acid analogs, puromycin	abnormal proteins

Stress response: cellular changes

- transcriptional upregulation of the synthesis of some genes

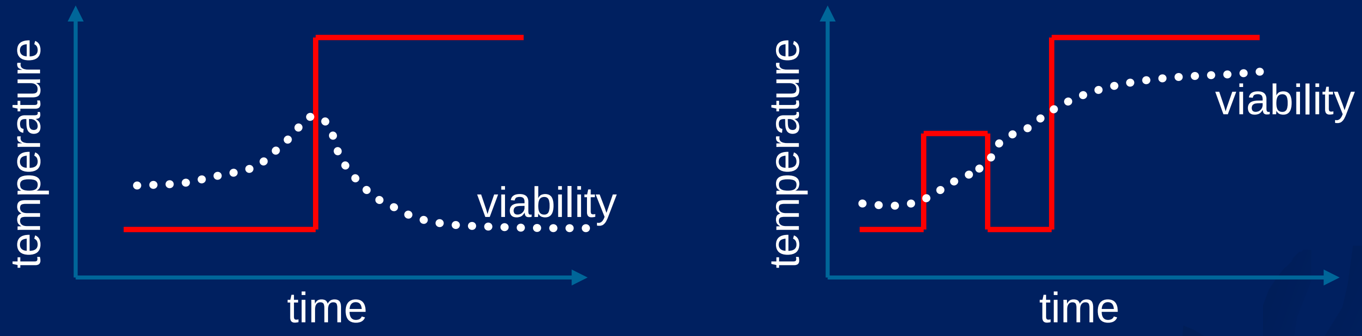
  - major proteins produced have the following sizes:

    <30, 40, 60, 70, 90, ~100 kDa

- downregulation of the production of most proteins
- most dramatic example:

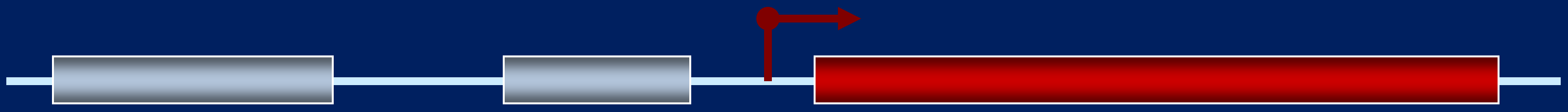
  - Pyrodictium occultum thermosome (chaperonin) accumulates to ~70% or more of total cellular protein; other proteins not produced

- *acquired thermotolerance*



Regulation of the stress response: prokaryotes

- stress-inducible genes have promoters that differ from genes that are not induced under stress conditions:



TCTCNCCCTTGAA **CCCCATNTA**

-35 HS promoter -10

stress-inducible gene(s)
- sometimes part of an operon, *e.g.*, GroEL-GroES or DnaK-DnaJ-GrpE

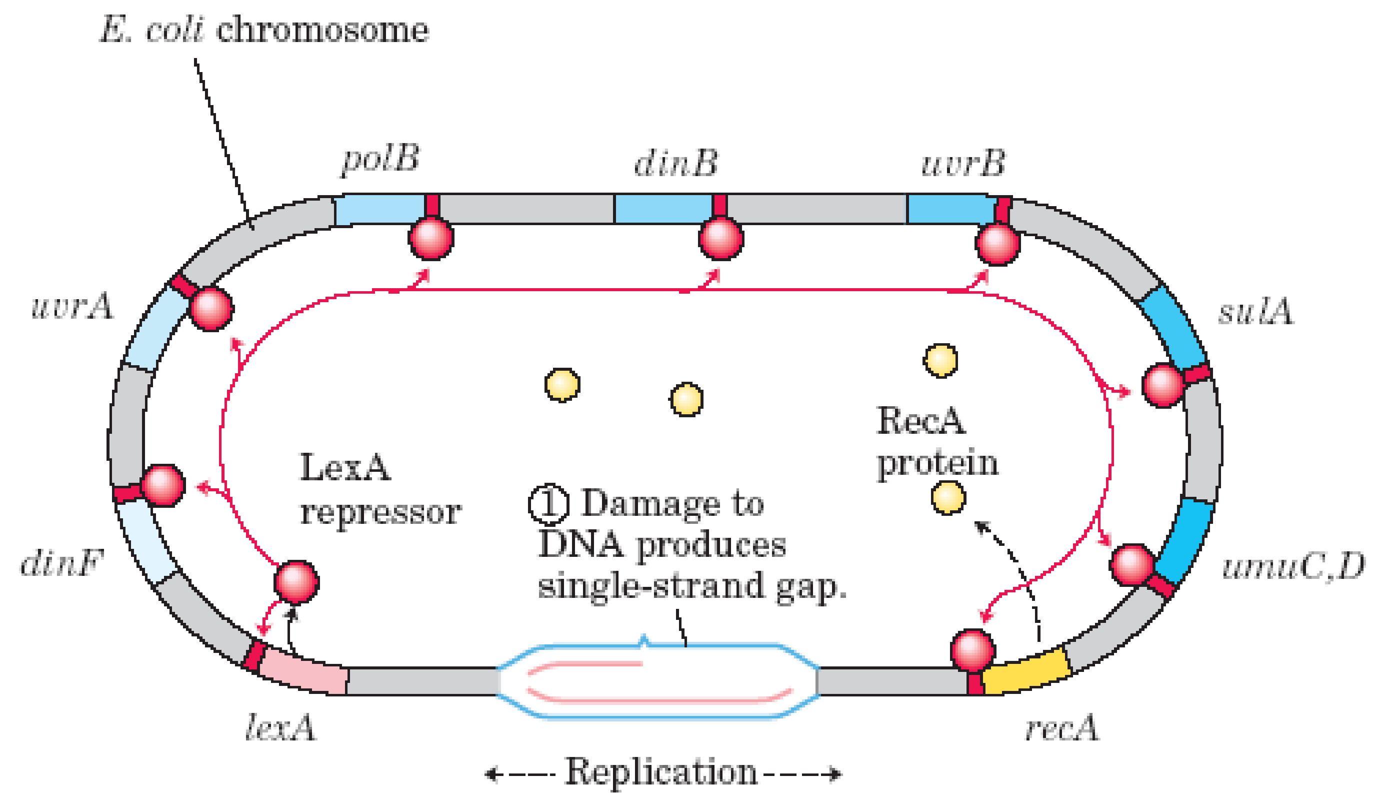
- σ^{70} (sigma-70) binds RNAP and activates transcription of genes
- σ^{32} (sigma-32) can also assemble with RNAP, but it is unstable and rapidly degraded under physiological conditons; under stress conditions (*e.g.*, heat-shock), it is stabilized and it assembles with RNAP
- RNAP-σ^{32} binds HS promoters, upregulating their transcription
- DnaK (Hsp70), DnaJ (Hsp40) and GrpE *E. coli* mutants have a constitutively active stress response

SOS response

SOS response: a cellular stress response to extensive DNA damage, i.e. exposure to strong UV light.

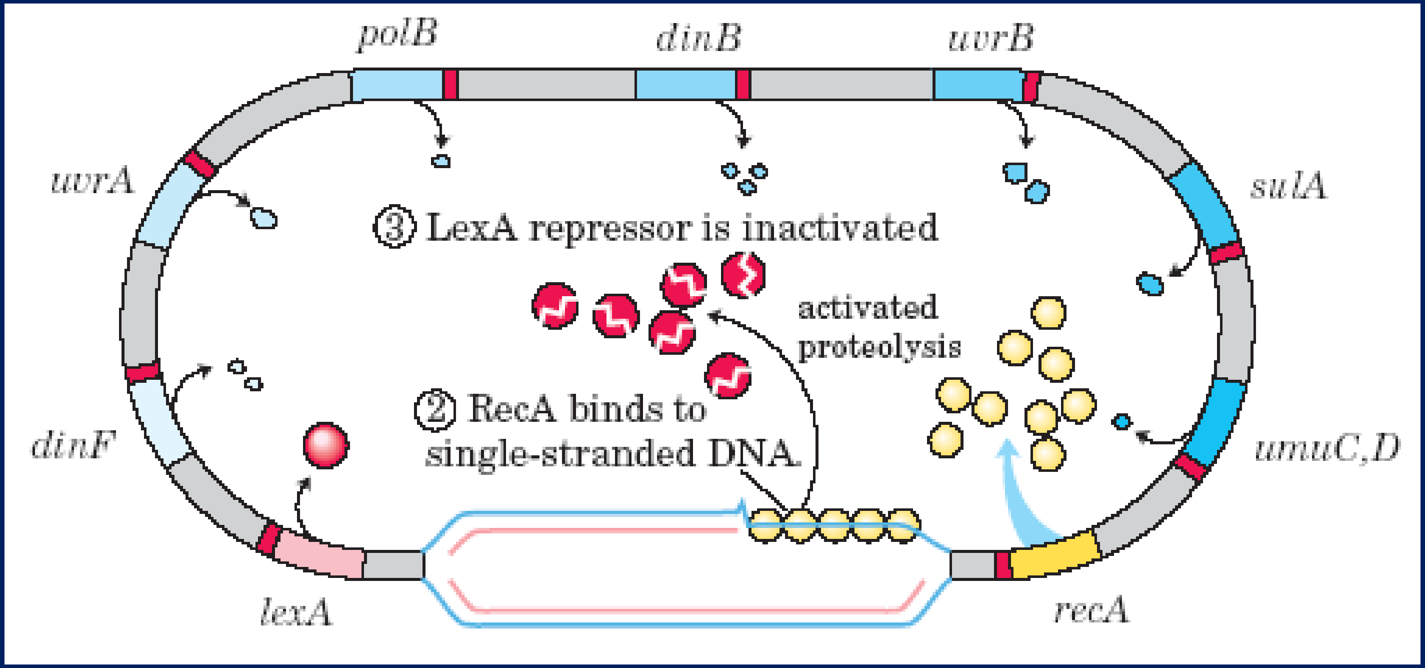
Heavy DNA damage halts replication. The SOS response is a desperation strategy to overcome a nearly insurmountable barrier to cell division.

SOS response



SOS response: a cellular stress response to extensive DNA damage. The LexA repressor protein inhibits transcription of all SOS genes, and induction of the SOS response requires removal of LexA.

LexA digestion


polB
dinB
uvrB
uvrA
sulA
③ LexA repressor is inactivated
activated proteolysis
② RecA binds to single-stranded DNA
dinF
umuC,D
lexA
recA

This is not a simple dissociation of LexA.

The LexA repressor is inactivated when it catalyzes its own *proteolytic cleavage* at a specific ala-gly peptide bond.

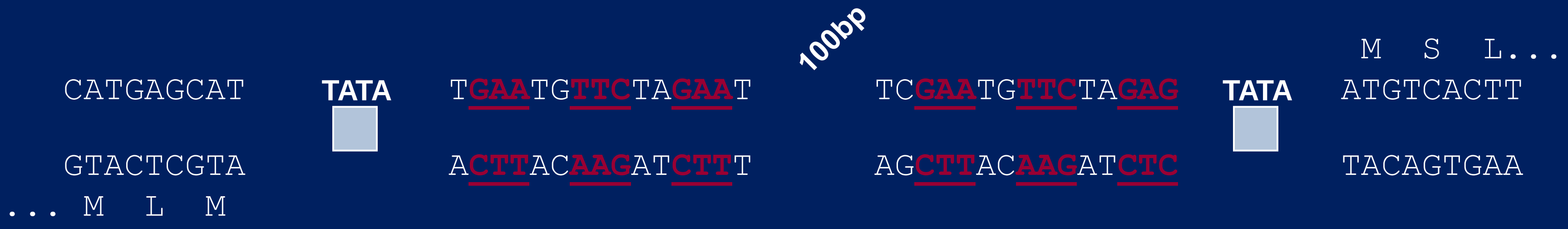
This cleavage requires a coprotease called RecA.

RecA facilitates cleavage of LexA only *when it is bound to single-stranded gaps in damaged DNA*.

Regulation of the stress response: eukaryotes

- *Heat-shock transcription factor (HSF) binds heat-shock elements (HSE).* The HSE has been conserved throughout evolution, from yeast to humans
- HSEs consist of the sequences nGAAn and its complement nTTCn, and occur in tandem (multiple copies)

  - an example of a potent, bi-directional *heat-shock promoter* is from the *C. elegans* Hsp16-1 and Hsp16-48 operon:

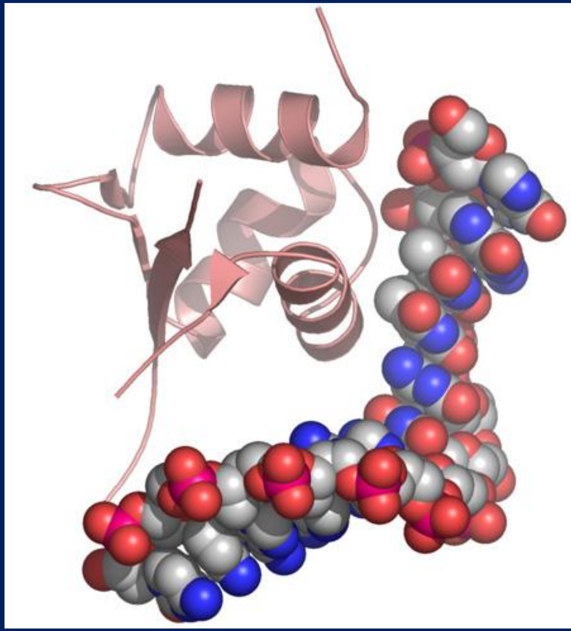


100bp
M S L...
CATGAGCAT **TATA** TGAATGTTCTAGAAT TCGAATGTTCTAGAG **TATA** ATGTCACTT
GTACTCGTA ACTTACAAGATCTTT AGCTTACAAGATCTC TACAGTGAA
... M L M

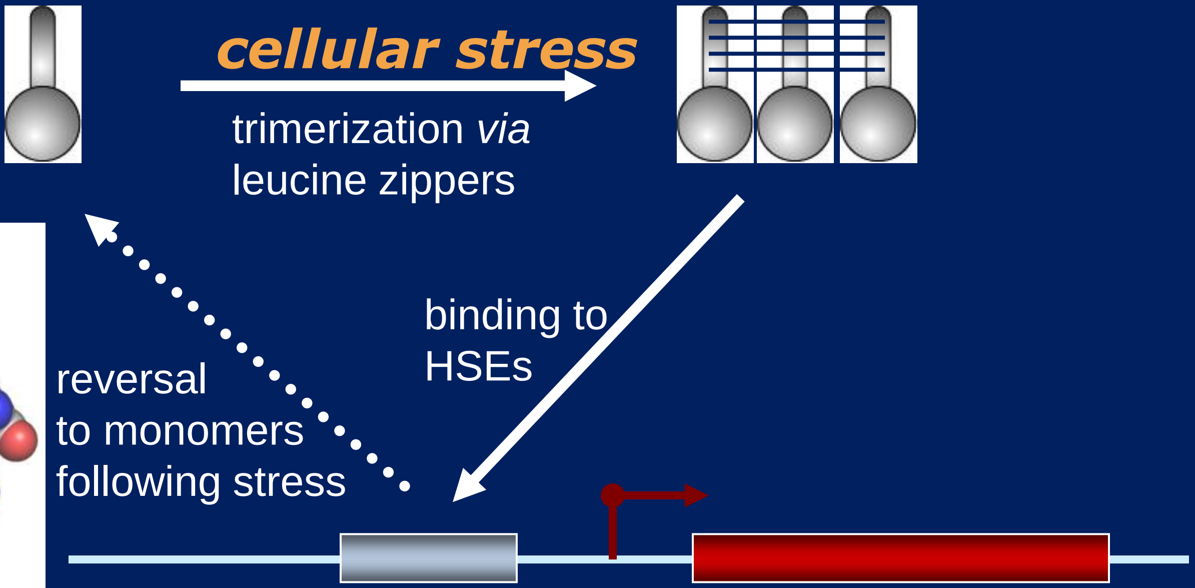
- at least 2-3 HSE sequences are required for HSF binding; HSF binding to HSEs is cooperative: the more HSEs present, the stronger the binding
- many heat-shock protein promoters have been used to control gene expression

Regulation of the stress response: HSF

HSF has a Winged Helix-Turn-Helix Motif



HSF DNA binding domain (monomer) in complex with HSE sequence



Inactive heat shock transcription factor (HSF) monomer

cellular stress

trimerization *via* leucine zippers

Active trimer

binding to HSEs

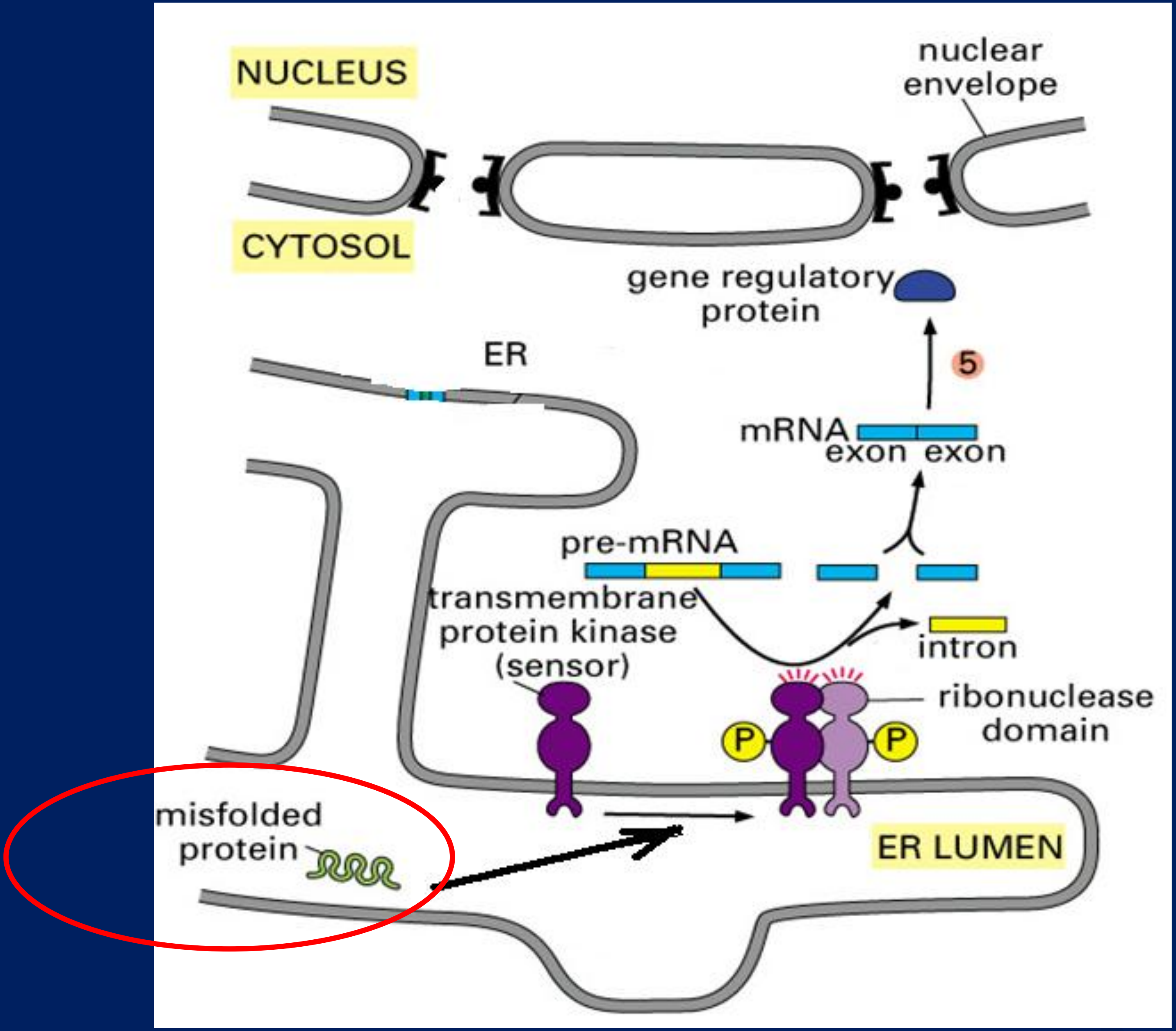
reversal to monomers following stress

HSEs

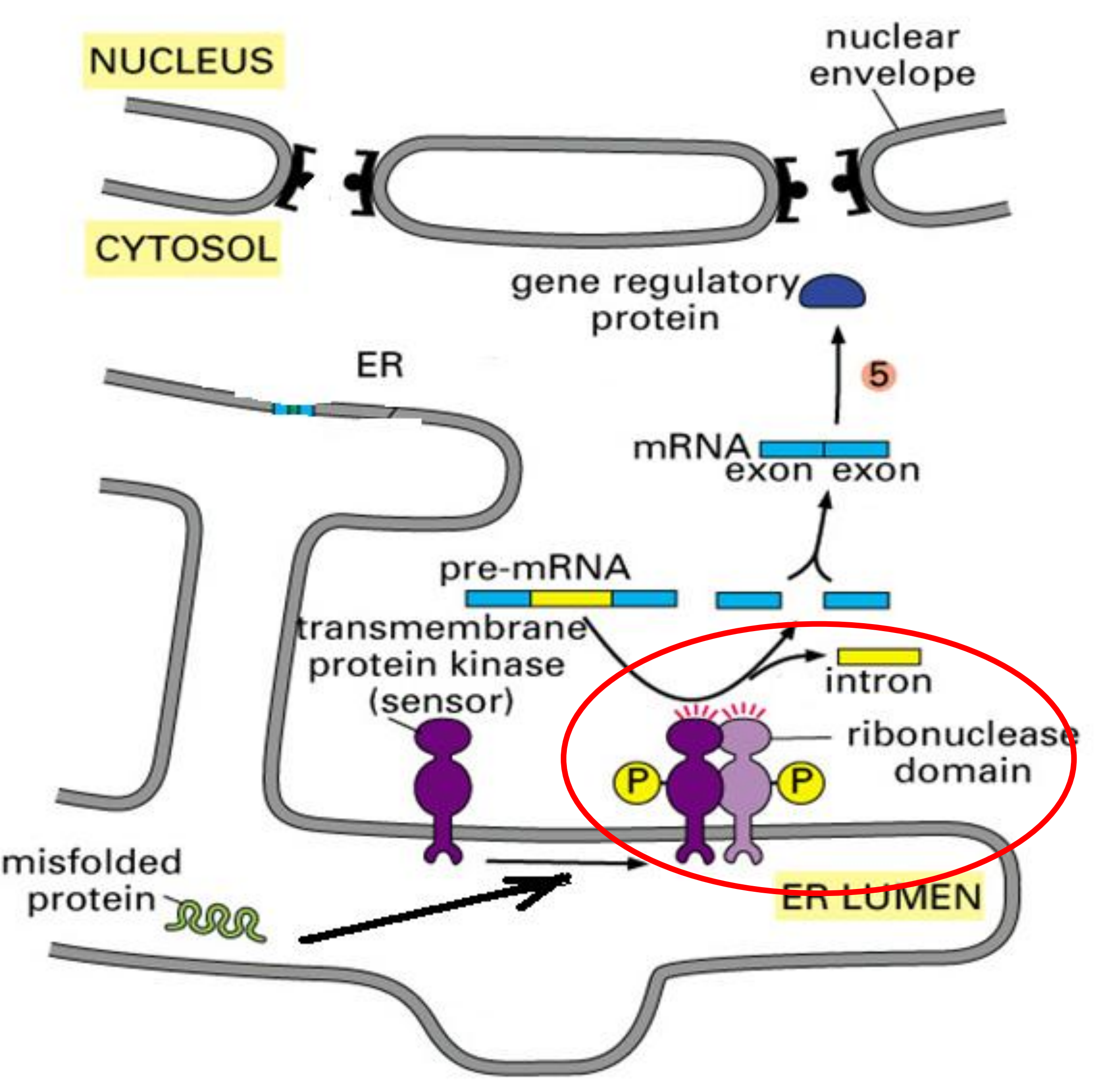
stress-inducible gene

- activation of transcription by HSF requires phosphorylation
- monomer-trimer transition, and activity while bound to DNA is regulated by molecular chaperones

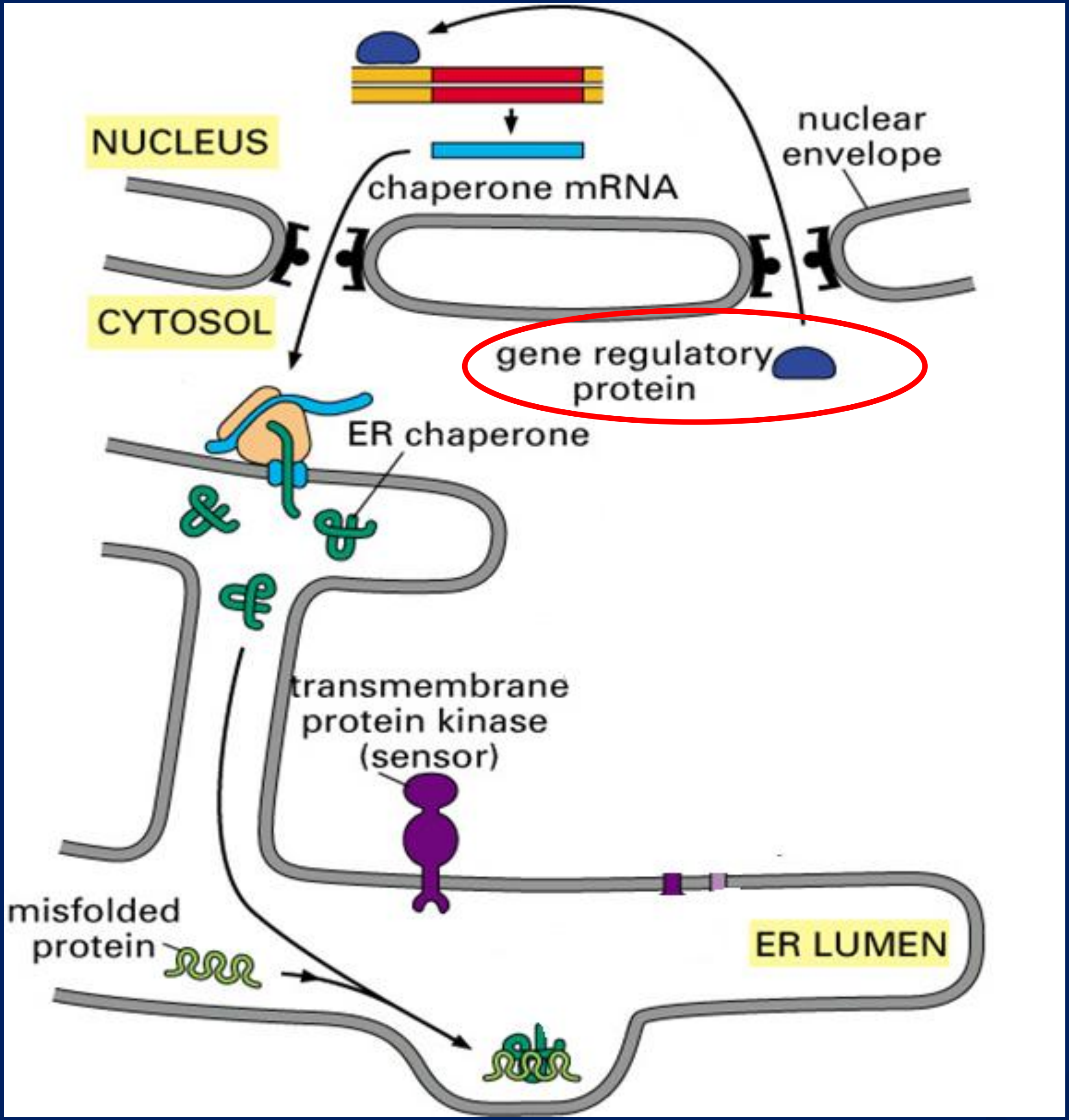
The Unfolded Protein Response (UPR)



The Unfolded Protein Response (UPR)



The Unfolded Protein Response (UPR)



The Unfolded Protein Response (UPR)

Hsp4
(grp78)
grp170

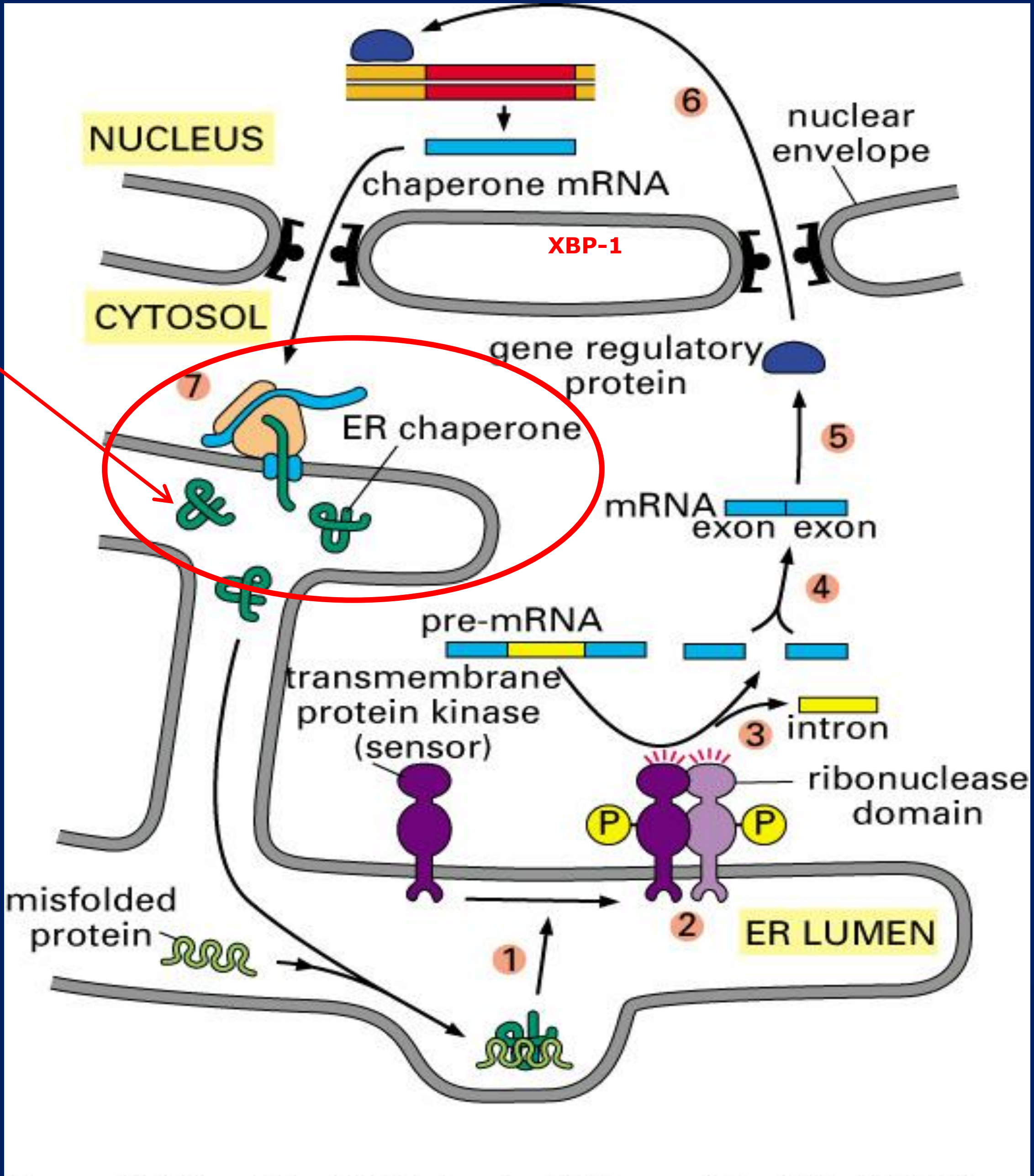
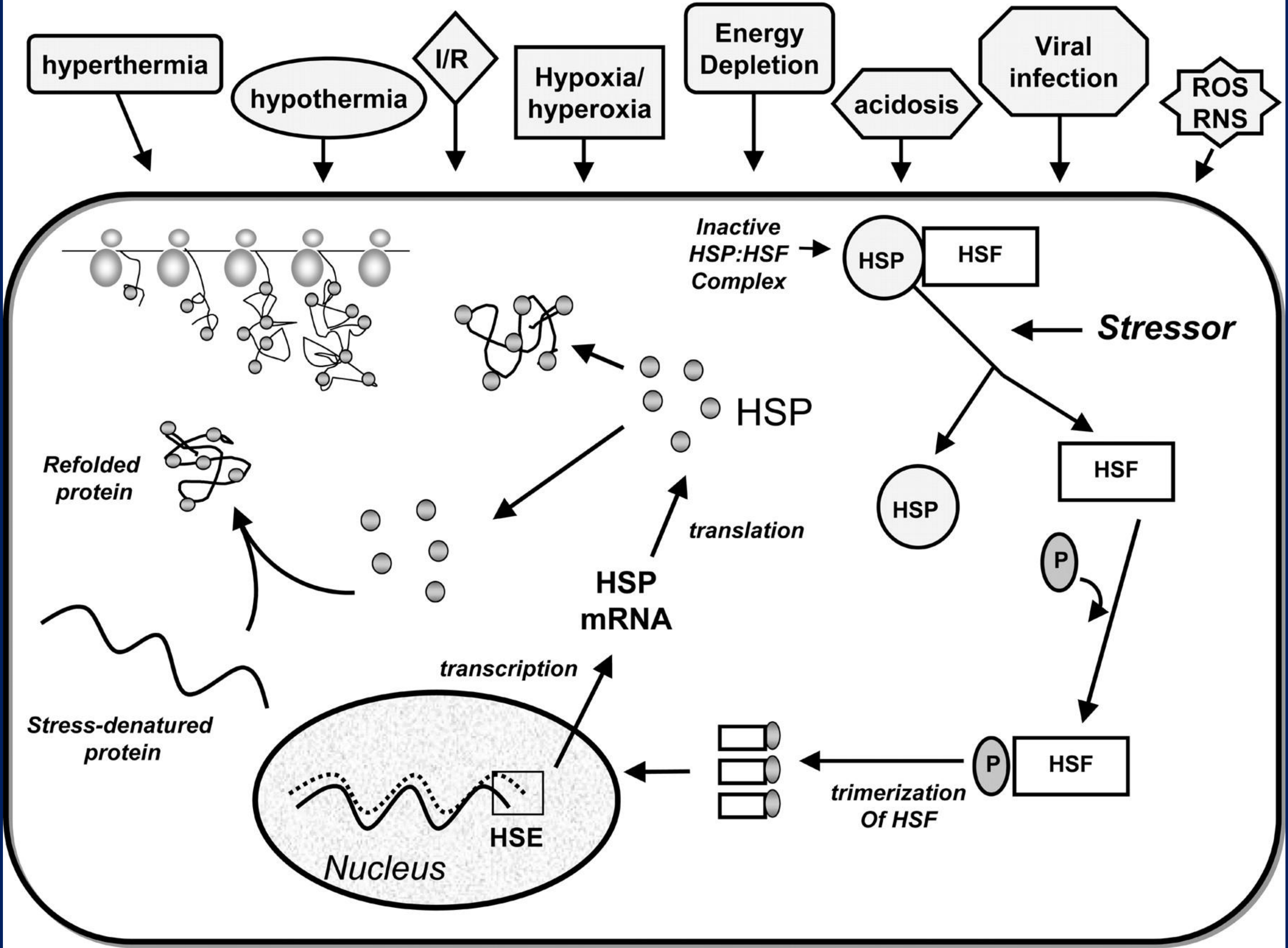


Figure 12–56 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

HSP Family	Cellular Location	Proposed Function
HSP27 (sHSP)	Cytosol, nucleus	Microfilament stabilization, antiapoptotic
HSP60	Mitochondria	Refolds proteins and prevents aggregation of denatured proteins, proapoptotic
HSP70 family:		Antiapoptotic
HSP72 (Hsp70)	Cytosol, nucleus	Protein folding, cytoprotection
HSP73 (Hsc70)	Cytosol, nucleus	Molecular chaperones
HSP75 (mHSP70)	Mitochondria	Molecular chaperones
HSP78 (GRP78)	ER	Cytoprotection, molecular chaperones
HSP90	Cytosol, ER, nucleus	Regulation of steroid hormone receptors, protein translocation
HSP110/104	Cytosol	Protein folding

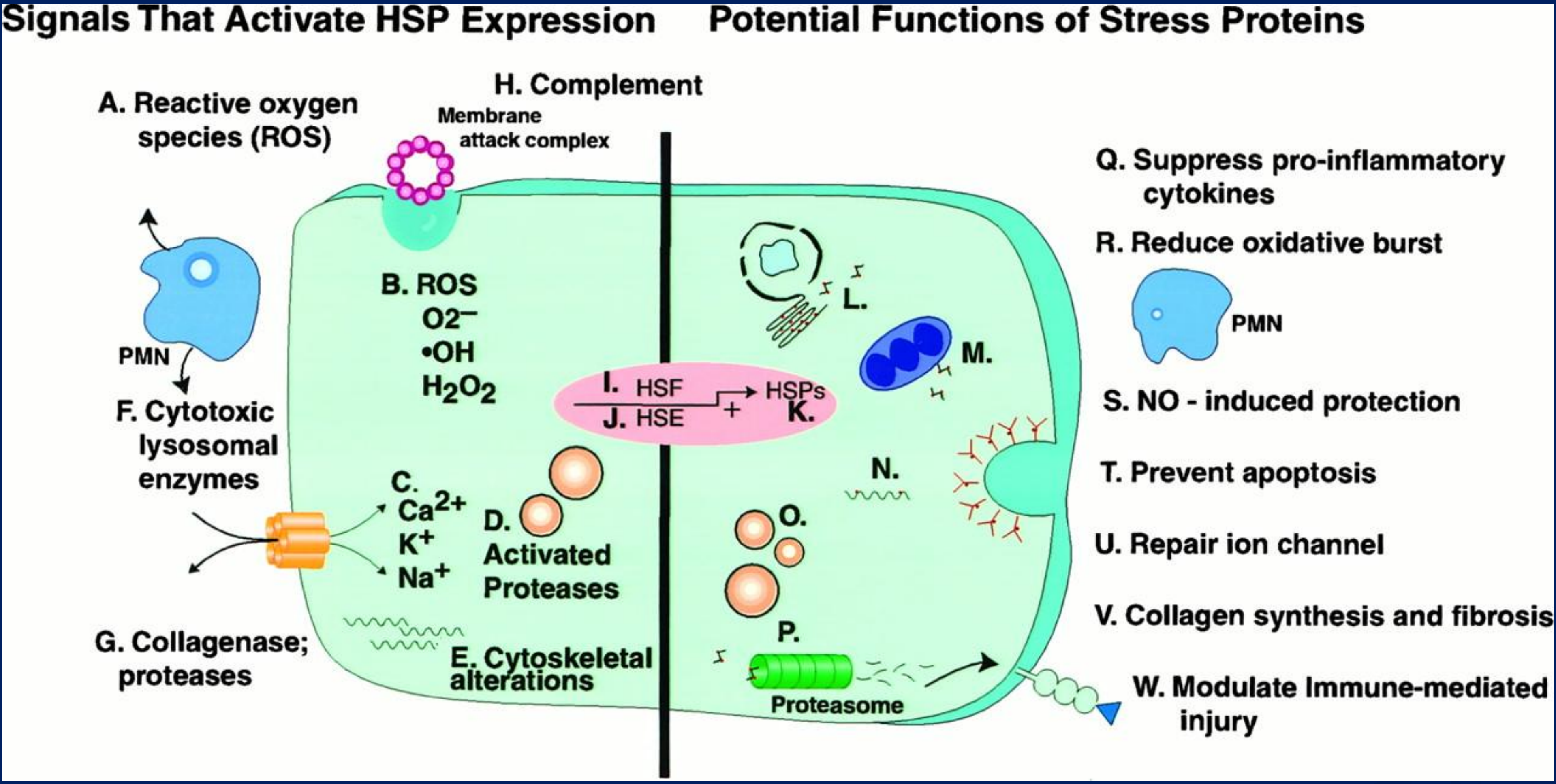
Table 1. Cellular locations and proposed functions of mammalian heat shock protein familiesHSP, heat shock protein; sHSP, small HSP; ER, endoplasmic reticulum

Physiological signals that activate HSP70 expression



Kregel, K. C. J Appl Physiol 92: 2177-2186 2002;

A summary of the major pathophysiological signals that activate HSP synthesis (left of vertical solid line) and the potential functions of HSPs (right of vertical solid line)


Signals That Activate HSP Expression
Potential Functions of Stress Proteins
H. Complement
Membrane attack complex
A. Reactive oxygen species (ROS)
PMN
F. Cytotoxic lysosomal enzymes
G. Collagenase; proteases
B. ROS
$O2^-$
•OH
H_2O_2
C.
Ca^{2+}
K^+
Na^+
D. Activated Proteases
E. Cytoskeletal alterations
I. HSF
J. HSE
+
HSPs
K.
L.
M.
N.
O.
P.
Proteasome
Q. Suppress pro-inflammatory cytokines
R. Reduce oxidative burst
PMN
S. NO - induced protection
T. Prevent apoptosis
U. Repair ion channel
V. Collagen synthesis and fibrosis
W. Modulate Immune-mediated injury

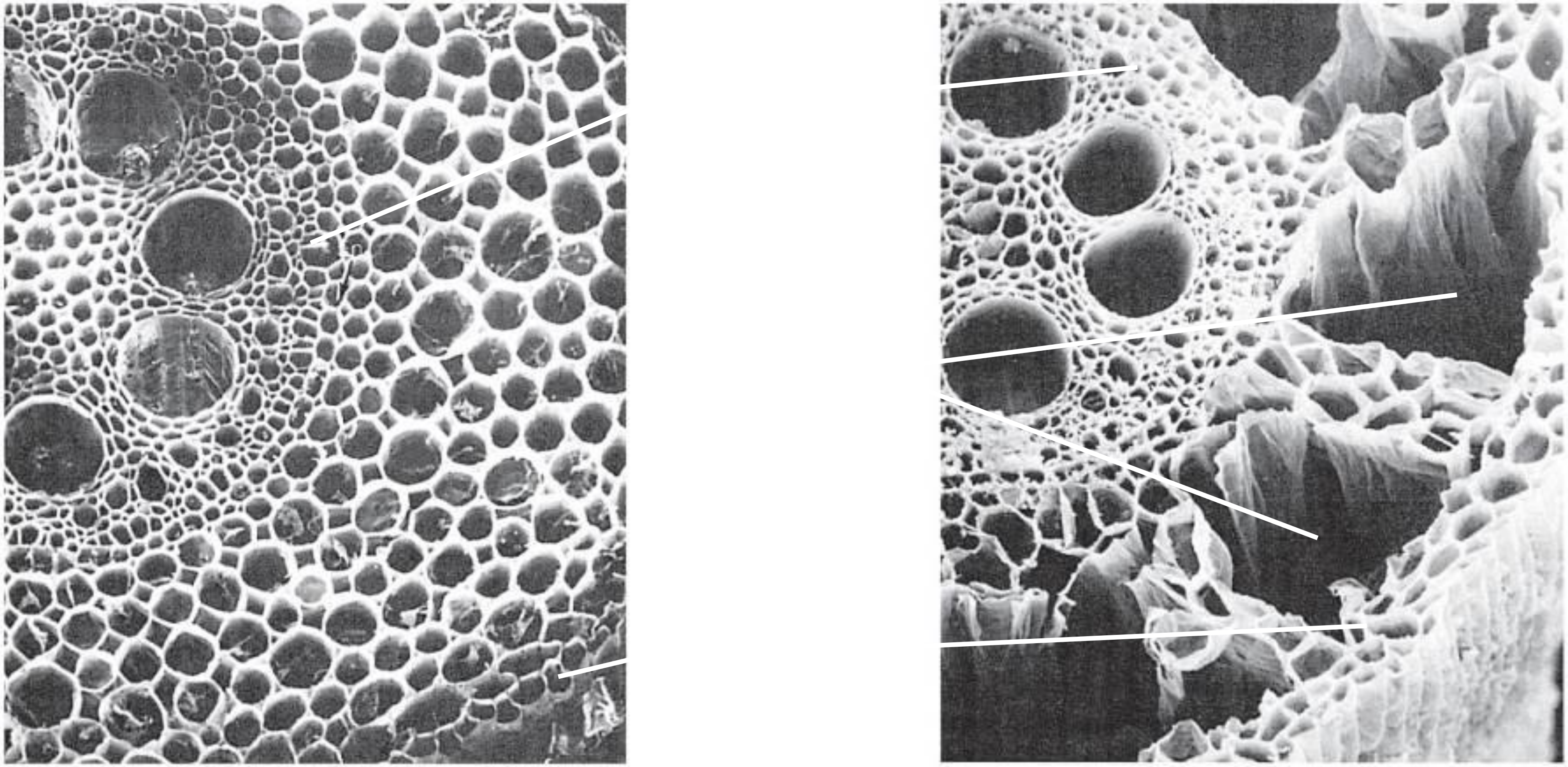
Benjamin, I. J. et al. Circ Res 1998;83:117-132

Environmental Stresses for Plants

- Environmental stresses have a potentially adverse effect on survival, growth, and reproduction
- Stresses can be **abiotic** (nonliving) or **biotic** (living)
- Abiotic stresses include drought, flooding, salt stress, heat stress, and cold stress

Drought and Flooding

- During drought, plants reduce transpiration by closing stomata, slowing leaf growth, and reducing exposed surface area
- Growth of shallow roots is inhibited, while deeper roots continue to grow
- Enzymatic destruction of root cortex cells creates air tubes that help plants survive oxygen deprivation during flooding



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Salt Stress

- Salt can lower the water potential of the soil solution and reduce water uptake
- Plants respond to salt stress by producing solutes tolerated at high concentrations
- This process keeps the water potential of cells more negative than that of the soil solution

Heat and Cold Stress

- Excessive heat can denature a plant’s enzymes
- **Heat-shock proteins** help protect other proteins from heat stress
- Cold temperatures decrease membrane fluidity
- Altering lipid composition of membranes is a response to cold stress
- Freezing causes ice to form in a plant’s cell walls and intercellular spaces

Активные формы кислорода и азота.
Антиоксидантные системы.
Redox- signaling

Prooxidants

Free Radicals:

- Any species capable of independent existence that contains one or more unpaired electrons
- A molecule with an unpaired electron in an outer valence shell

$R_3C\cdot$	Carbon-centered
$R_3N\cdot$	Nitrogen-centered
$R\text{-}O\cdot$	Oxygen-centered
$R\text{-}S\cdot$	Sulfur-centered

Non-Radicals:

- Species that have strong oxidizing potential
- Species that favor the formation of strong oxidants (e.g., transition metals)

H_2O_2	Hydrogen peroxide
$HOCl^-$	Hypochlorous acid
O_3	Ozone
$^1O_2$	Singlet oxygen
$ONOO^-$	Peroxynitrite
Me^{n+}	Transition metals

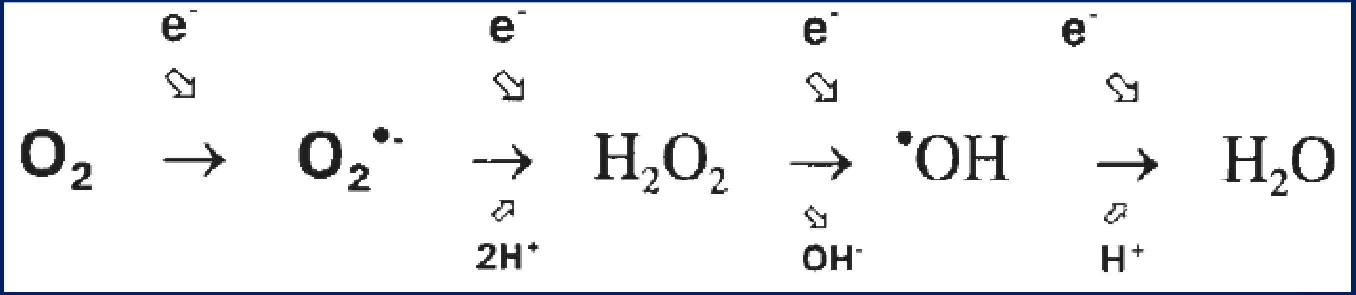
Types of Free Radicals

- Oxygen-centered radicals
  - Singlet oxygen, superoxide, hydroxyl radicals
- Sulfur-centered radicals
  - Thiyl radical (RS•)
- Carbon-centered radicals
  - $\cdot CCl_3$, $CH_2\cdot CHOH$
- Nitrogen-centered radicals
  - $NO\cdot$, $R_2NO\cdot$

Oxygen-Centered Radicals

- Reactive Oxygen Species (ROS)

Radicals	**Non-Radicals**
Superoxide, $O_2^{\bullet -}$	Hydrogen peroxide, H_2O_2
Hydroxyl, $HO^{\bullet}$	Hypochlorous acid, HOCl
Peroxyl, $ROO^{\bullet}$	Ozone, O_3
Alkoxyl, $RO^{\bullet}$	Singlet oxygen, $^1\Delta g$
Hydroperoxyl, $HOO^{\bullet}$	Peroxynitrite, $ONOO^-$



Superoxide Radicals

- Generation of superoxide ($O_2^{\cdot -}$)
  - The addition a single electron to the ground-state molecule ($O_2 + e^- \rightarrow O_2^{\cdot -}$)
- Biological generation of $O_2^{\cdot -}$
  - Mitochondrial electron transport chain
  - Enzymatic reduction of oxygen (O_2)
  - Xenobiotic metabolisms (redox cycling)
  - Respiratory burst (phagocytes)

Hydroxyl Radical ($HO^{\cdot}$)

- **Highly reactive oxygen radicals**
- Formation of hydroxyl radicals in biological systems
  - Ionizing radiation
  - Reaction of metal ions with hydrogen peroxide (Fenton reaction)
  - Formation of hydroxyl radical from ozone (O_3)
- Reactions of hydroxyl radicals
  - Hydrogen atom abstraction
  - Addition
  - Electron transfer

Fenton Reaction

- Discovered by Fenton (1894)
- “A mixture of hydrogen peroxide and an iron(II) salts causes the formation of **hydroxyl radical**”

$Fe^{2+} + H_2O_2 \longrightarrow$ intermediate complex $\longrightarrow Fe^{3+} + OH^- + HO\bullet$

$Fe^{3+} + H_2O_2 \longrightarrow$ intermediate complex $\longrightarrow Fe^{2+} + O_2^{\bullet -} + 2H^+$

- **Haber-Weiss reaction**

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + HO\bullet$$

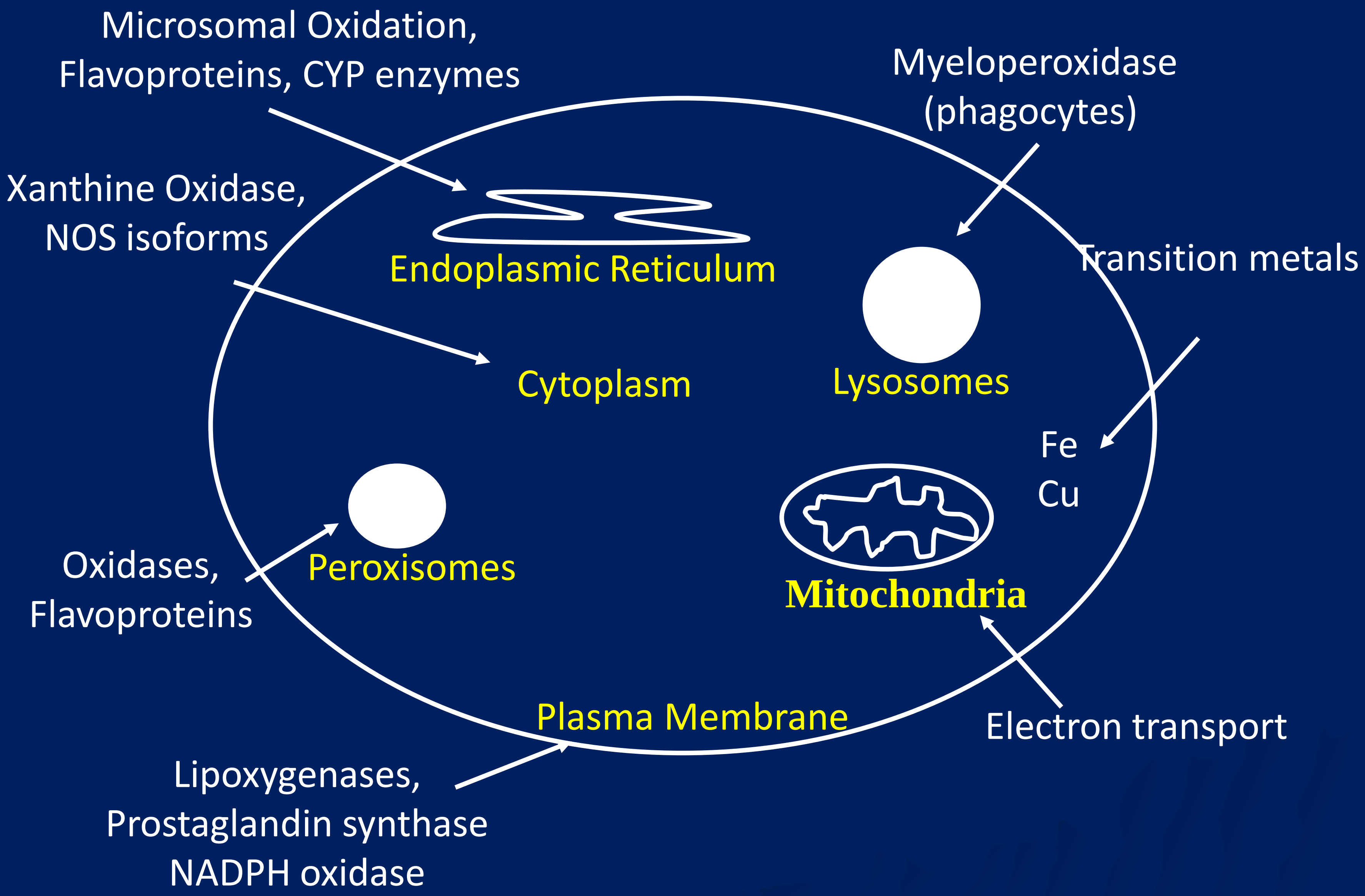
$$Fe^{3+} + O_2^{\bullet -} \longrightarrow Fe^{2+} + O_2$$

$$\text{Net : } O_2^{\bullet -} + H_2O_2 \xrightarrow[\text{catalyst}]{\text{metal}} O_2 + HO\bullet + OH^-$$

Nitrogen-Centered Radicals

- Nitric oxide ($NO^{\bullet}$)
  - Endothelial derived-relaxing factor (EDRF)
  - Generated from the catalysis of L-arginine by **nitric oxide synthase (NOS)** enzymes
  - Functions
    - Vascular function, platelet aggregation, immune response, neurotransmitter, signal transduction
    - cytotoxicity
  - $NO^{\bullet} + O_2^{\bullet -} \longrightarrow ONOO^{-}$ (highly toxic)

Endogenous sources of ROS and RNS



Microsomal Oxidation,
Flavoproteins, CYP enzymes

Myeloperoxidase
(phagocytes)

Xanthine Oxidase,
NOS isoforms

Endoplasmic Reticulum

Transition metals

Cytoplasm

Lysosomes

Fe
Cu

Oxidases,
Flavoproteins

Peroxisomes

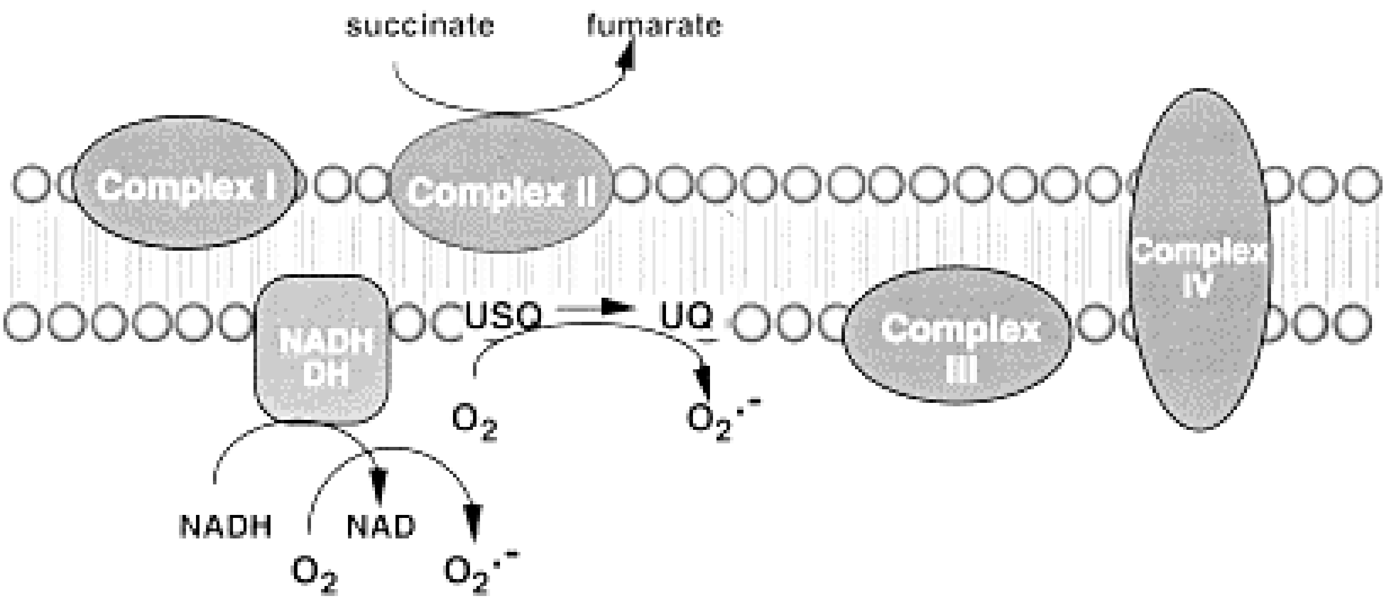
Mitochondria

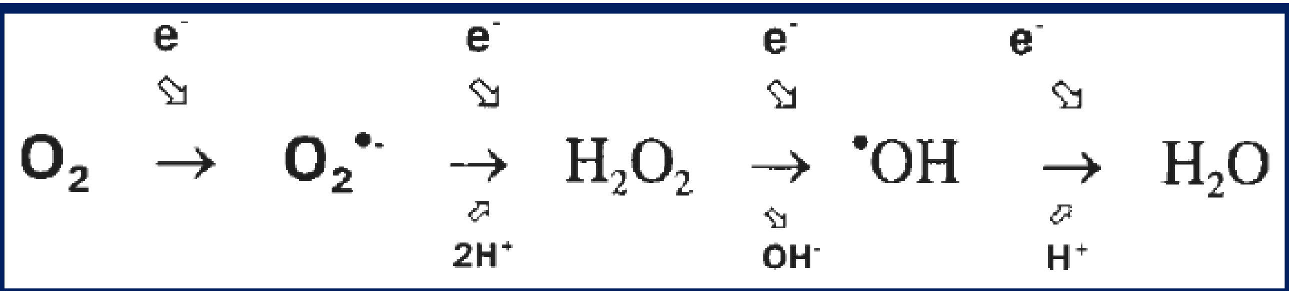
Plasma Membrane

Electron transport

Lipoxygenases,
Prostaglandin synthase
NADPH oxidase

Mitochondria as a source of ROS



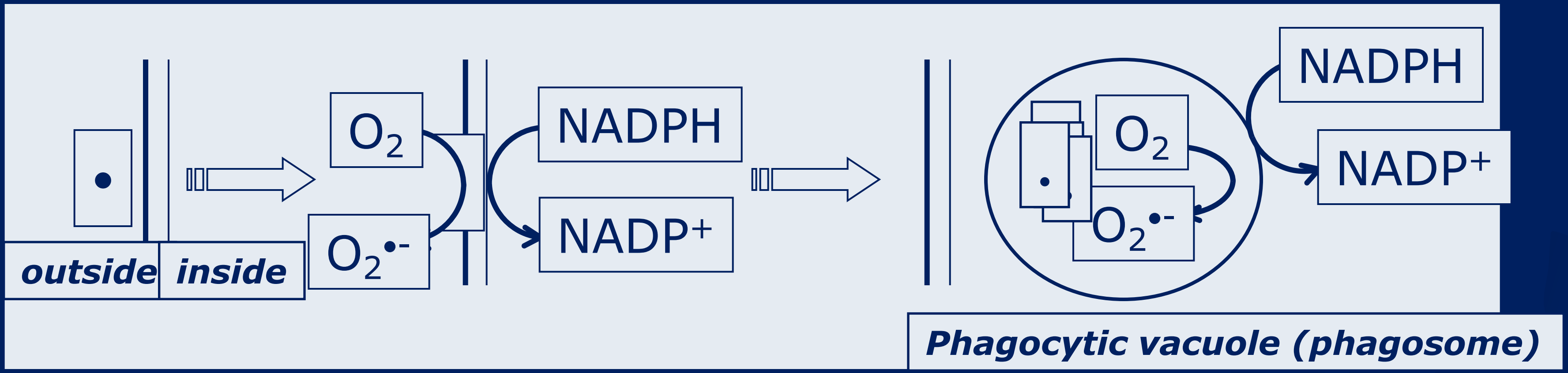


Complex I (NADH-coenzyme Q) and, to a lesser extent, following the auto-oxidation of coenzyme Q from the Complex II (succinate-coenzyme Q) and/or Complex III (coenzyme QH2-cytochrome c reductases) sites. The precise contribution of each site to total mitochondrial ROS production is probably determined by local conditions including chemical or physical damage to the mitochondria, oxygen availability and the presence of xenobiotics.

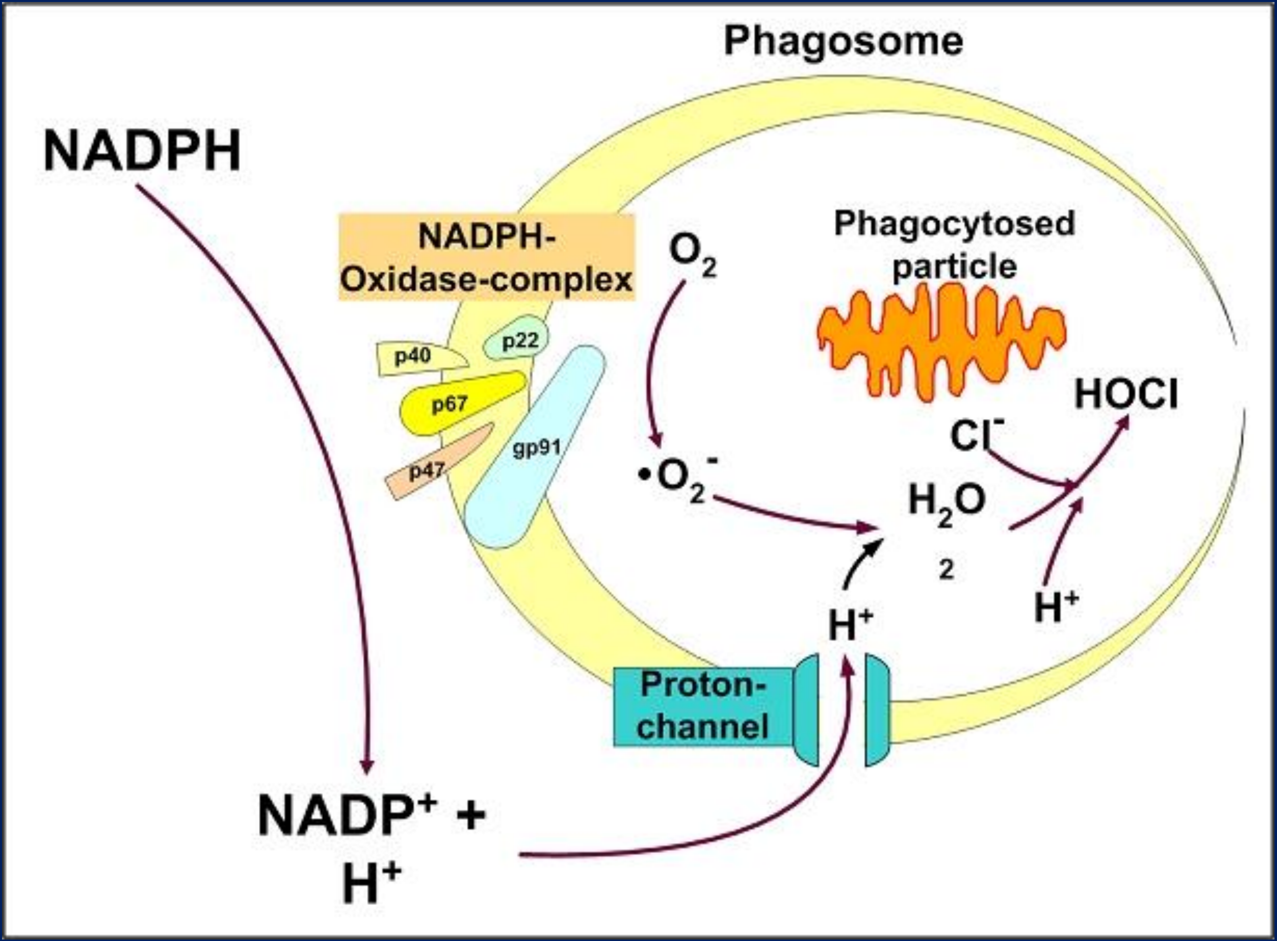
From Kehrer JP (2000) Toxicology 149: 43-50

Respiratory Burst

- Myeloperoxidase
  - Oxidizes Cl^- to hypochlorous acid
  - Chronic granulomatous disease
- NADPH oxidase enzyme



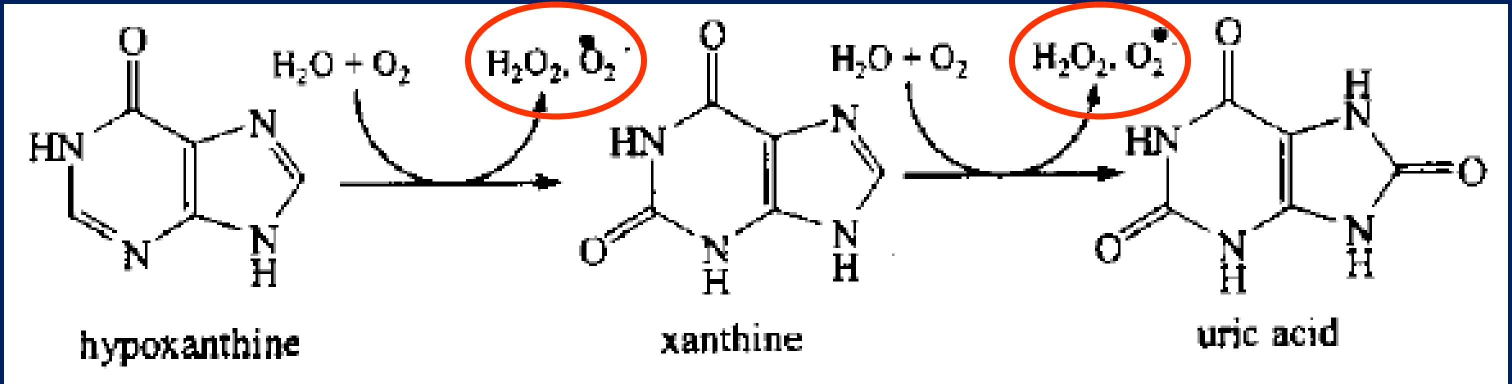
NADPH oxidase complex



- Cytoplasmic proteins (p47, p67, gp91, p22)
- NADPH → $NADP^+ + H^+$
- Electron is transferred from NADPH to O_2, resulting

in the formation of $O_2^{\bullet -}$

Cytoplasmic sources of ROS



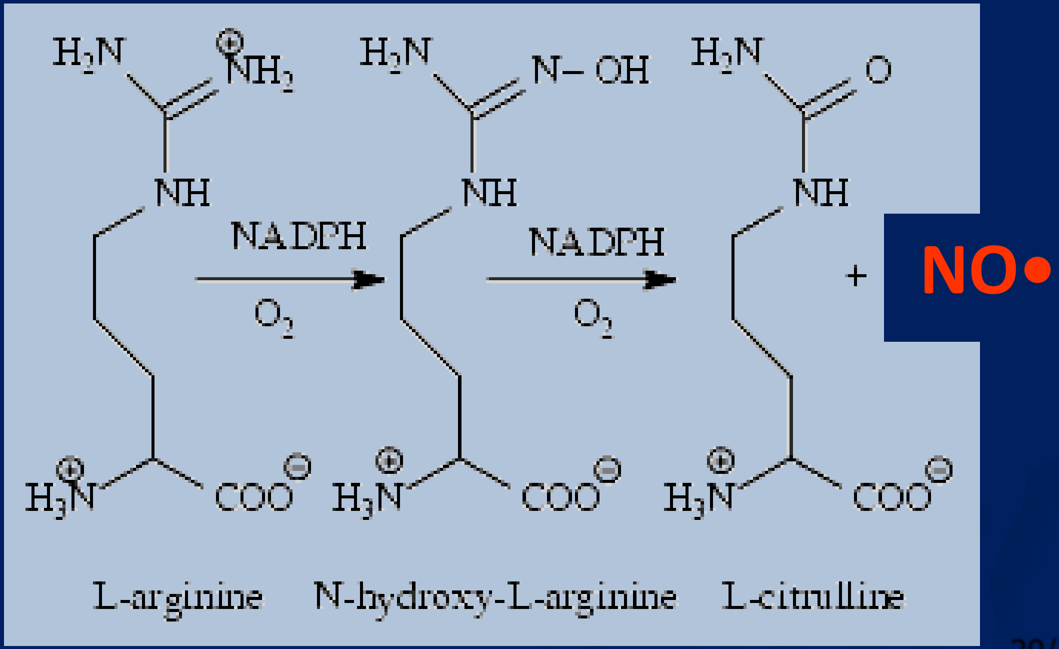
xanthine oxidase xanthine oxidase

Nitric Oxide Synthases (NOS):

neuronal nNOS (I)

endothelial eNOS (III)

inducible iNOS (II)

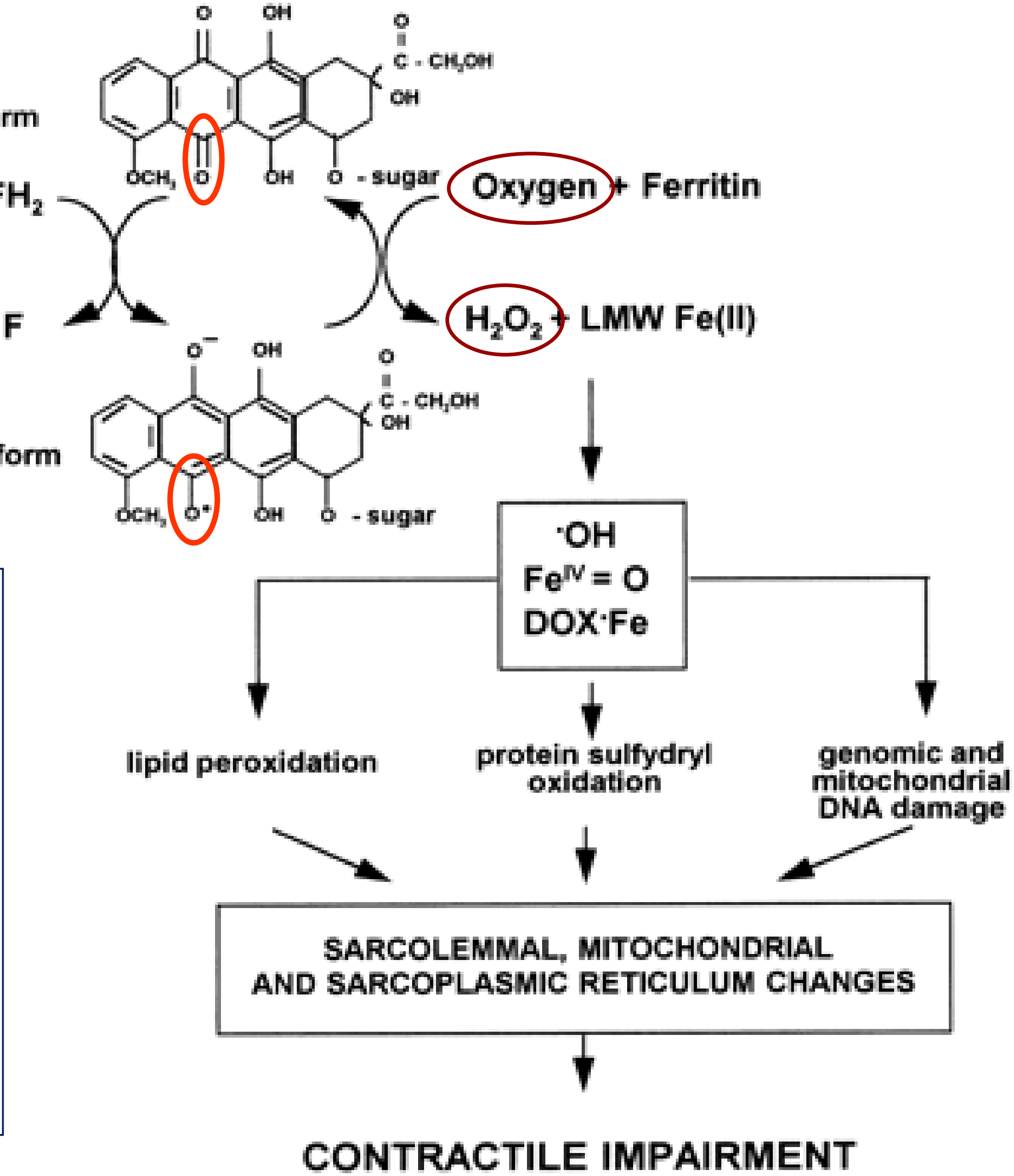


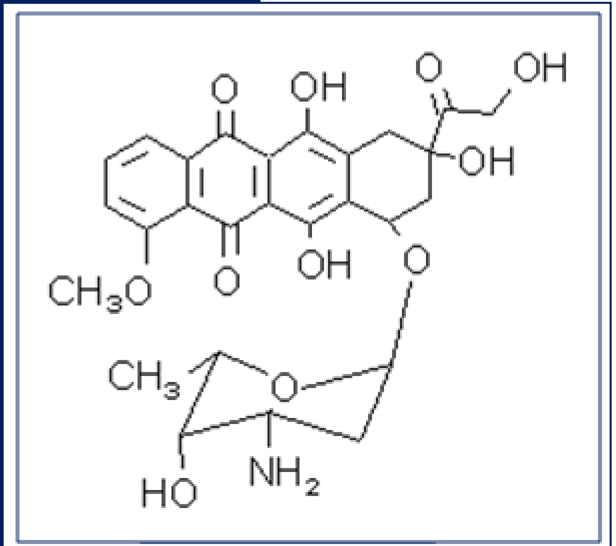
Exogenous sources of free radicals

- **Physical factors: radiation,** Ultrasound
  (UV light, x-rays, gamma rays)
- **Chemicals that react to form peroxides**
  Ozone and singlet oxygen
- **Chemicals that promote superoxide formation**
  Quinones, nitroaromatics, bipyrimidiulium herbicides
- **Chemicals that are metabolized to radicals**

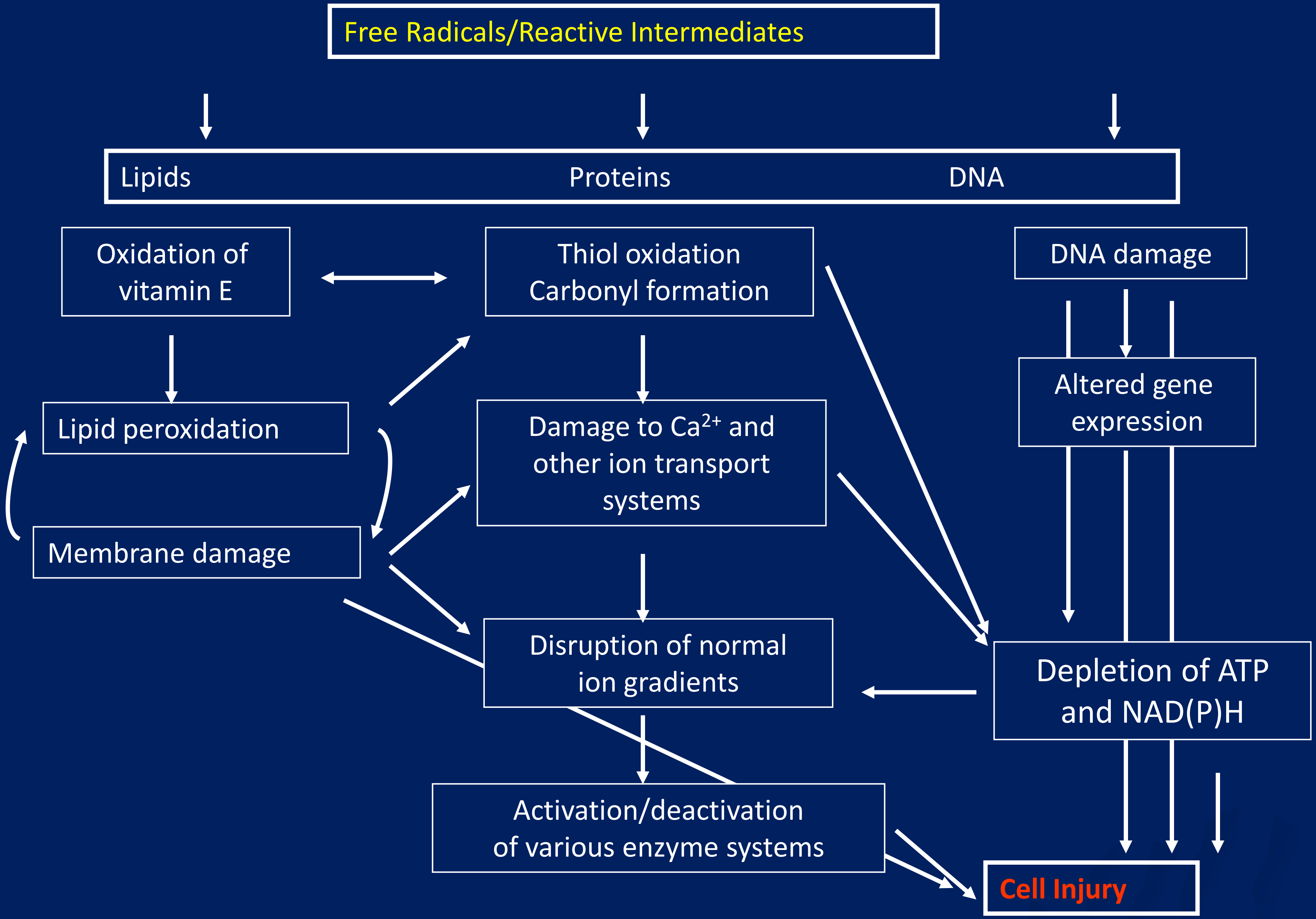
  e.g., polyhalogenated alkanes, phenols, aminophenols
- **Chemicals that release iron**
  ferritin

Drug-Induced Toxicity





Doxorubicin



Free Radicals/Reactive Intermediates

Lipids Proteins DNA

Oxidation of vitamin E

Thiol oxidation
Carbonyl formation

DNA damage

Lipid peroxidation

Damage to Ca^{2+} and other ion transport systems

Altered gene expression

Membrane damage

Disruption of normal ion gradients

Depletion of ATP and NAD(P)H

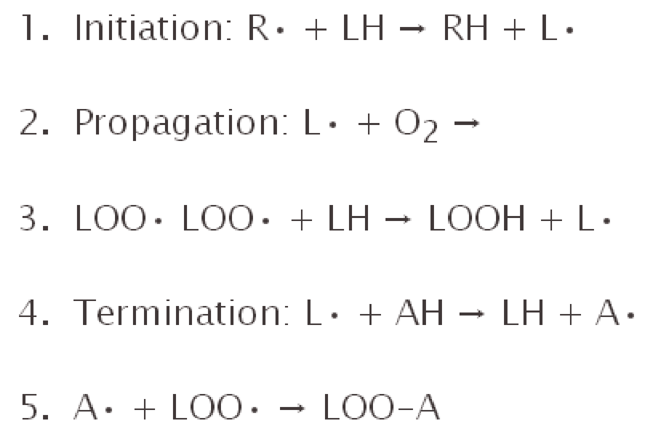
Activation/deactivation of various enzyme systems

Cell Injury

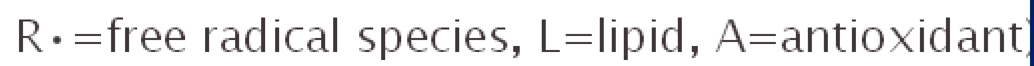
Adapted from: Kehrer JP, 1993

Lipid Peroxidation

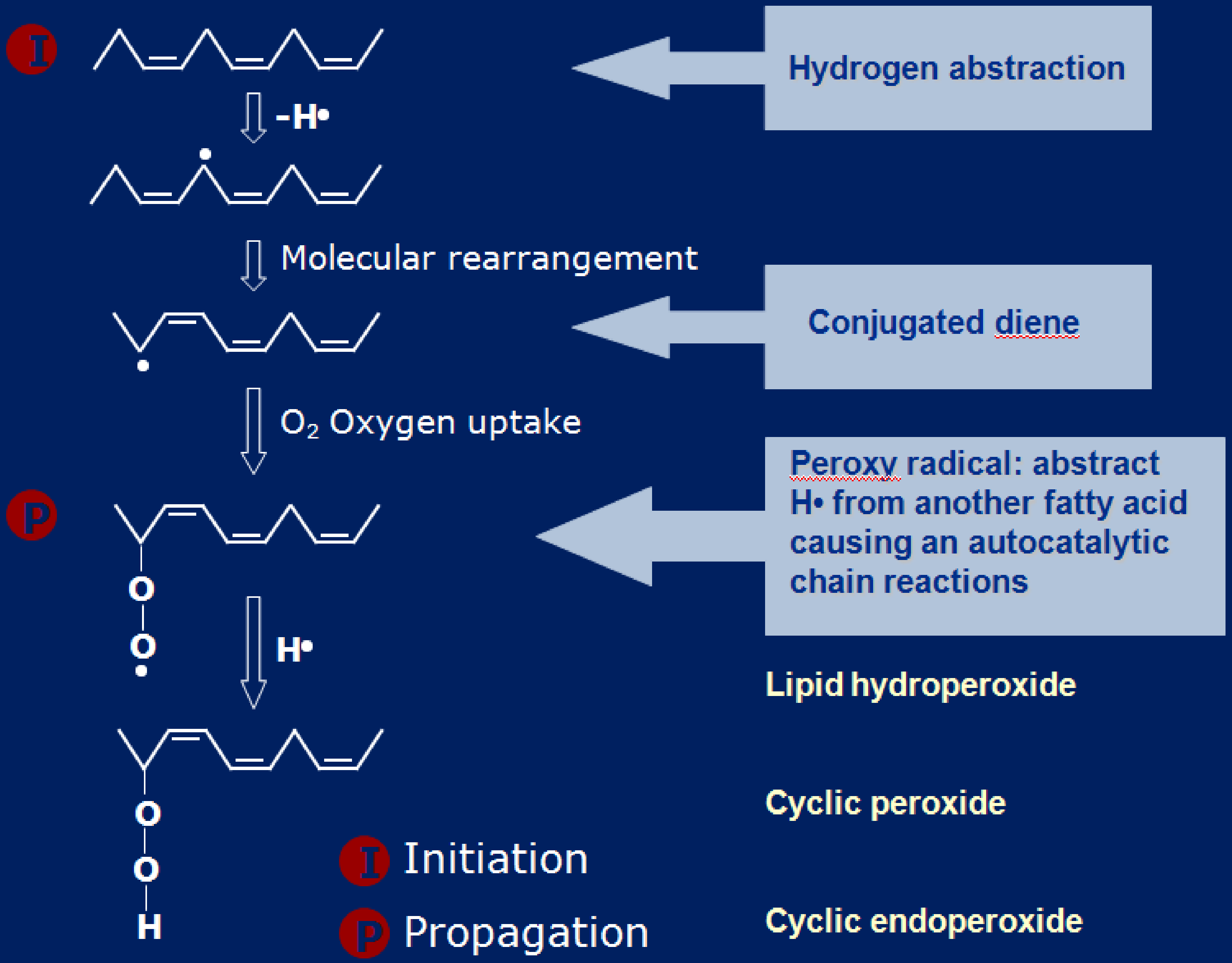
1. Initiation of first-chain reaction
    - Abstraction of H^+ by ROS (${}^{\bullet}OH$)
    - Formation of lipid radical ($LH^{\bullet}$)
    - Formation of peroxyl radical ($LOO^{\bullet}$, $ROO^{\bullet}$)
2. Propagation
    - H^+ abstraction by lipid peroxyl radical ($LOO^{\bullet}$)
3. Termination
    - Radical interaction → non-radical product



1. Initiation: $R\cdot + LH \rightarrow RH + L\cdot$
2. Propagation: $L\cdot + O_2 \rightarrow$
3. $LOO\cdot$ $LOO\cdot + LH \rightarrow LOOH + L\cdot$
4. Termination: $L\cdot + AH \rightarrow LH + A\cdot$
5. $A\cdot + LOO\cdot \rightarrow LOO{-}A$



$R\cdot$=free radical species, L=lipid, A=antioxidant



Hydrogen abstraction

Conjugated diene

Peroxy radical: abstract H• from another fatty acid causing an autocatalytic chain reactions

Initiation

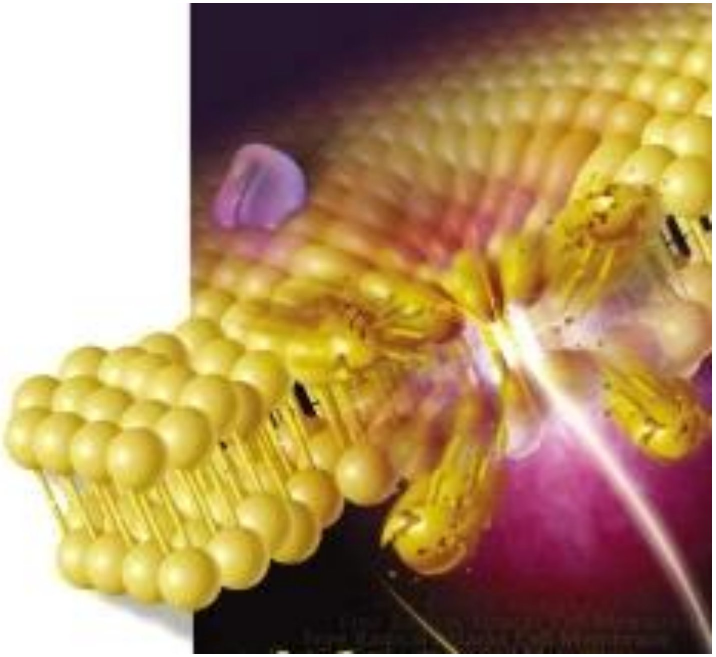
Propagation

Lipid hydroperoxide

Cyclic peroxide

Cyclic endoperoxide

Products of Lipid Peroxidation



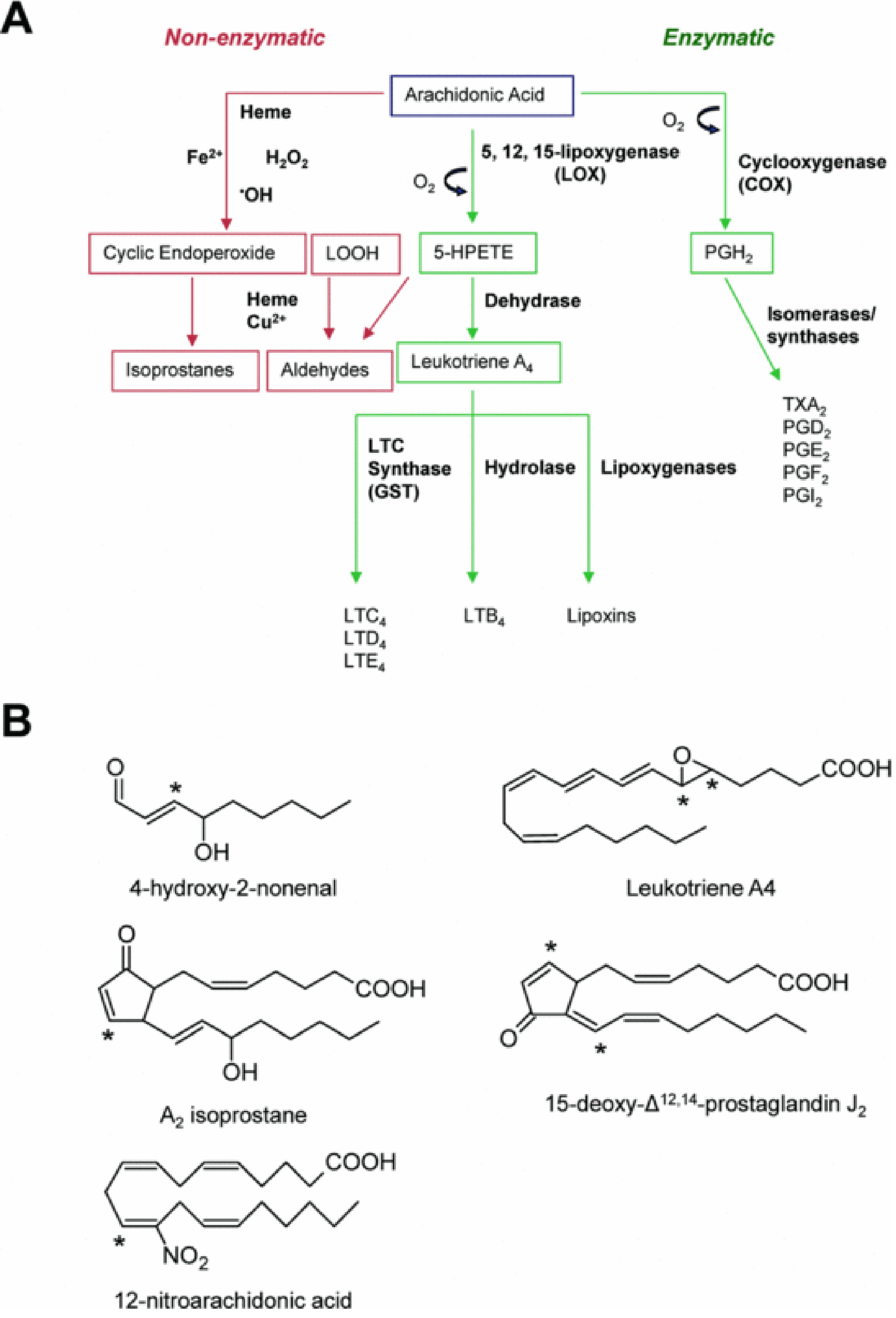
Reactive Oxygen Species

- Lipid peroxides
  - Conjugated dienes
  - Alkanes
  - Aldehyde products
    - Malondialdehyde (MDA)
    - *n*-aldehydes
    - α,β-unsaturated aldehydes

Measurement of Oxidative Stress

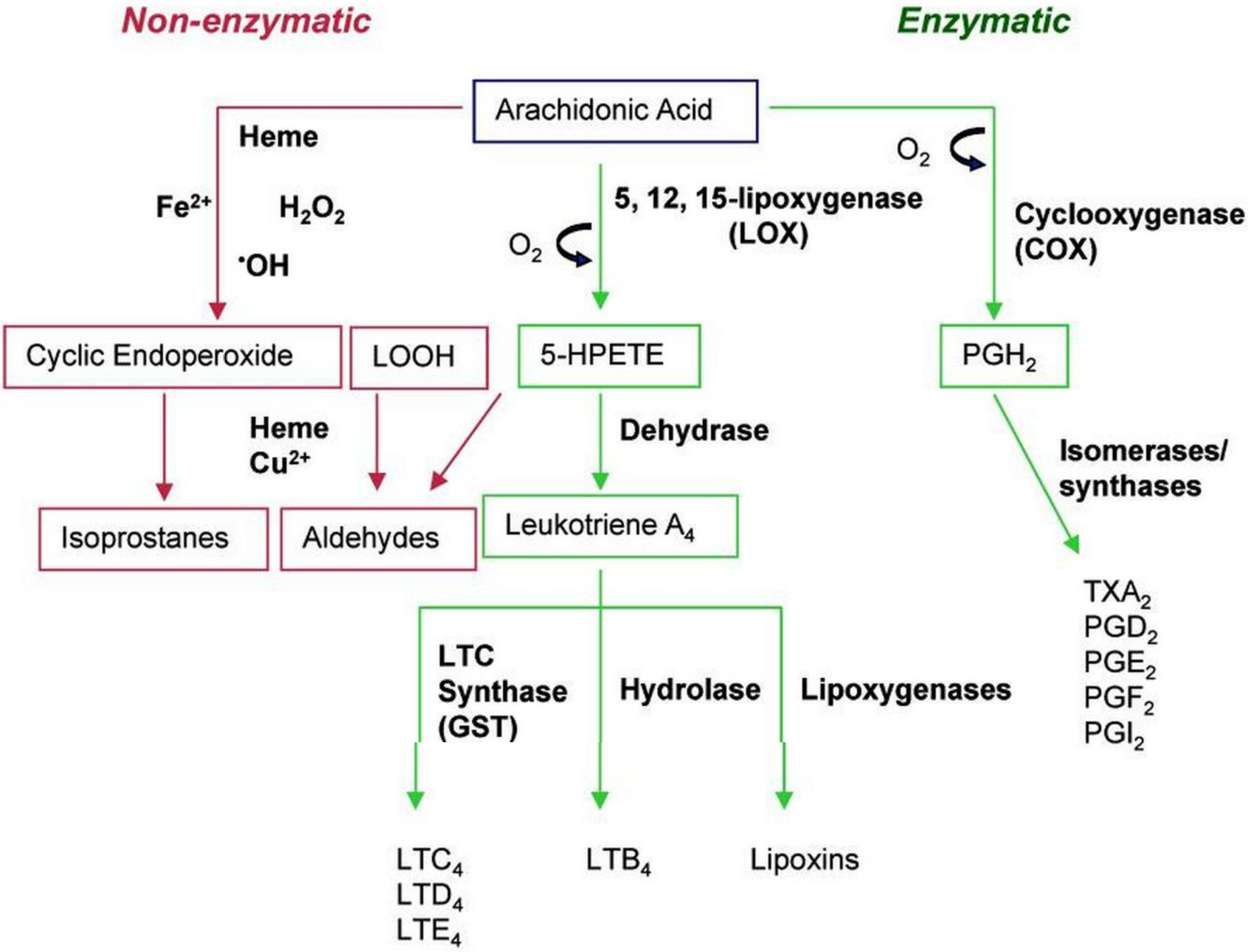
- **Oxygen consumption**
- **Oxidative markers “footprints”**
  - Lipid peroxidation products (TBARs, lipid hydroperoxides, etc)
  - DNA hydroxylation products (8-OHGua,
  - Protein hydroxylation products (nitrosation products)
- **Free radical detection**
  - Single photon counting
  - Chemiluminescence
  - Fluorescent probe
  - Electron paramagnetic resonance spectroscopy (EPR)

Formation of lipid electrophiles via non-enzymatic and enzymatic lipid peroxidation



Biochemical Journal 2012 442, 453-464 - Ashlee Higdon, Anne R. Diers and others.

www.biochemj.org



Non-enzymatic

Enzymatic

Arachidonic Acid

Heme

Fe^{2+}

H_2O_2

$^{\bullet}OH$

Cyclic Endoperoxide

LOOH

Heme
Cu^{2+}

Isoprostanes

Aldehydes

O_2

5, 12, 15-lipoxygenase
(LOX)

5-HPETE

Dehydrase

Leukotriene A_4

LTC
Synthase
(GST)

Hydrolase

Lipoxygenases

LTC_4
LTD_4
LTE_4

LTB_4

Lipoxins

O_2

Cyclooxygenase
(COX)

PGH_2

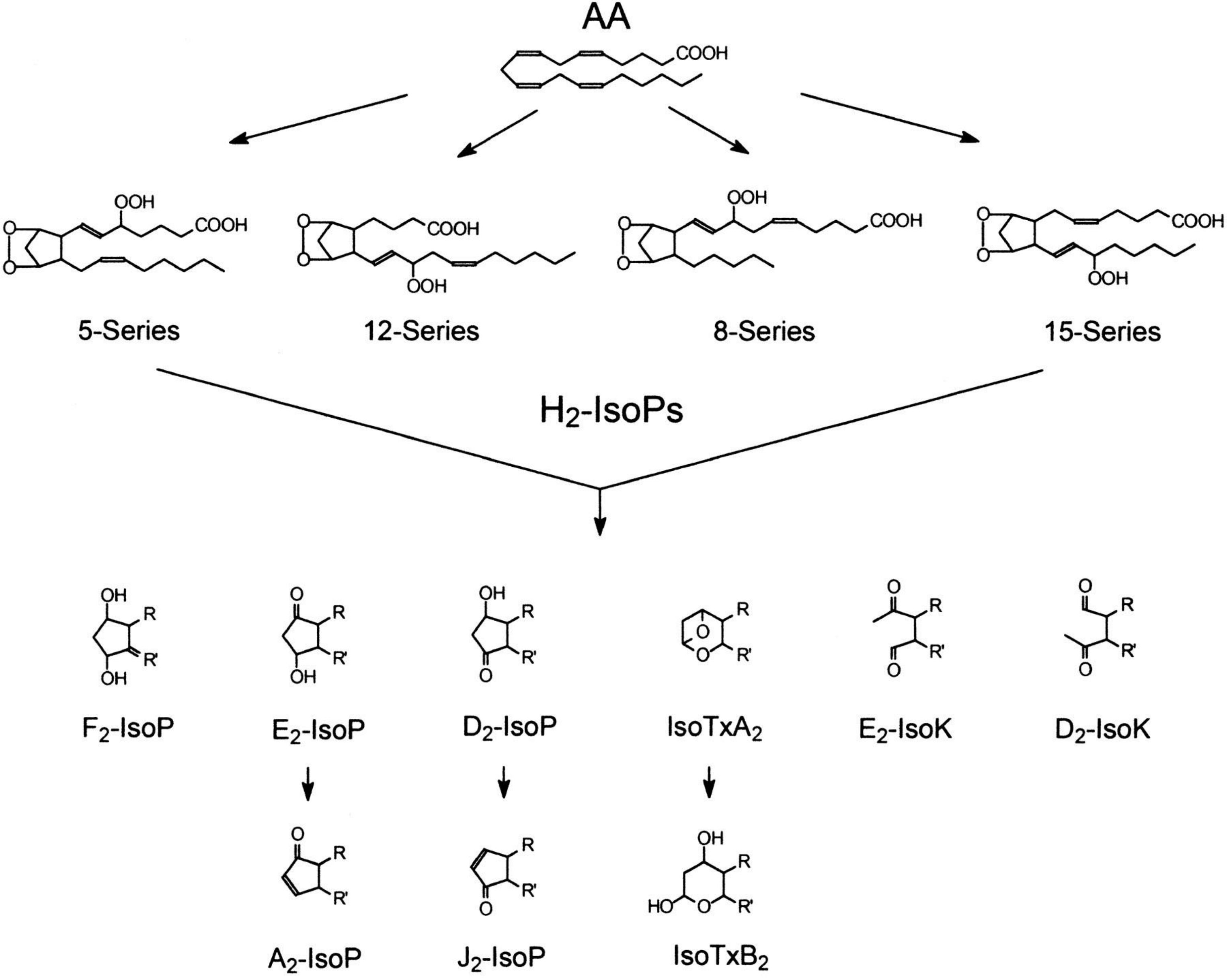
Isomerases/
synthases

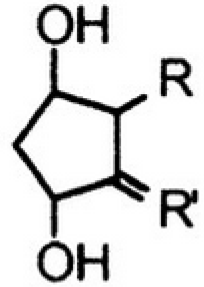
TXA_2
PGD_2
PGE_2
PGF_2
PGI_2

$PGF_{2\alpha}$-Isoprostane

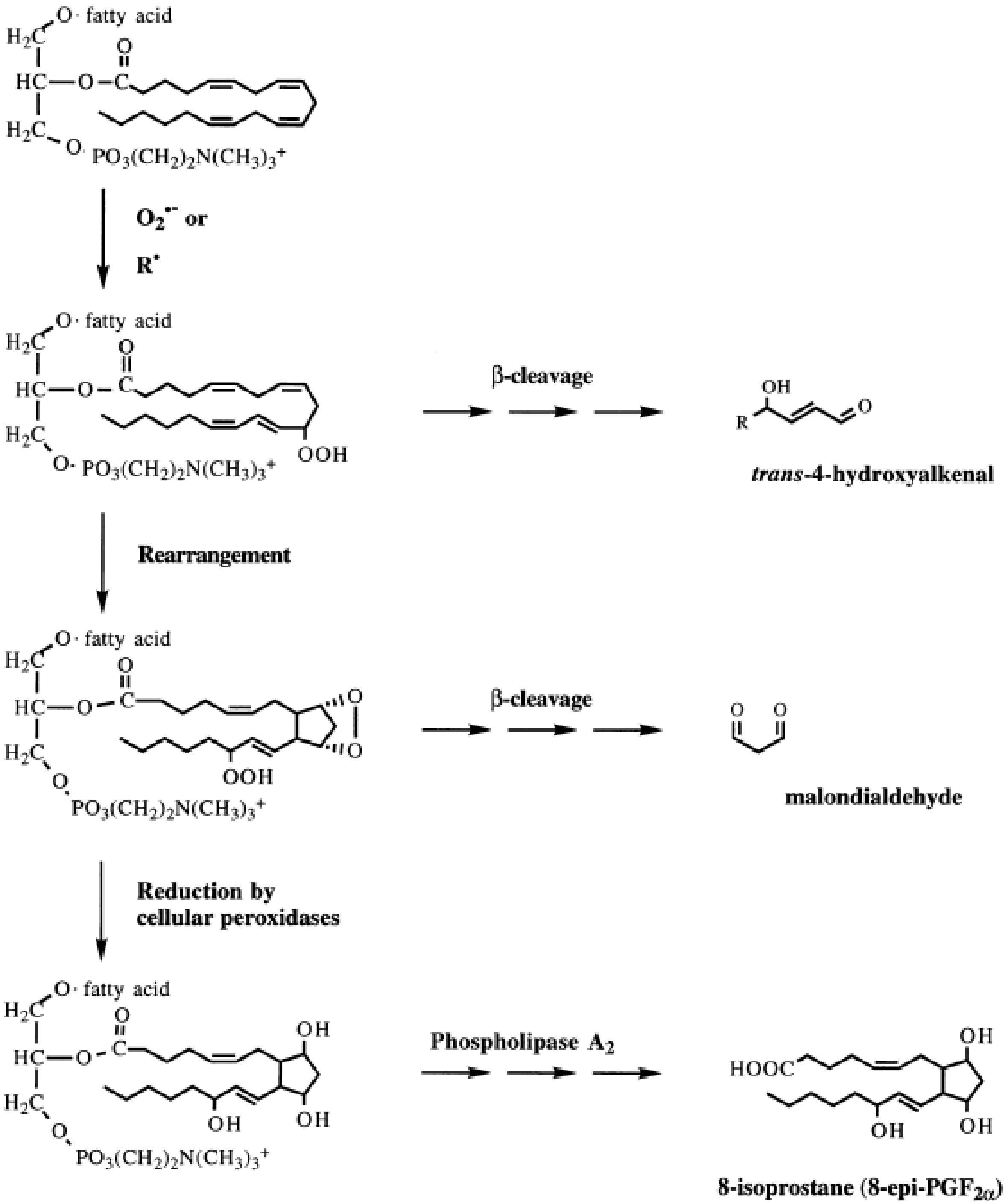
- A group of prostaglandin (PGF_2)-like compounds produced by non-enzymatic free radical-induced peroxidation of arachidonic acid
- Reliable biomarker of oxidative stress in vivo
  - Specific product of lipid peroxidation
  - Stable compound
  - Detectable level in biological samples
  - Level increases during oxidative injury
  - Formation is modulated by antioxidant status
  - Not affected by lipid contents from diet

Isoprostane (IsoP) pathway of free radical-induced oxidation of arachidonic acid. Tx: thromboxane. IsoK: isoketals





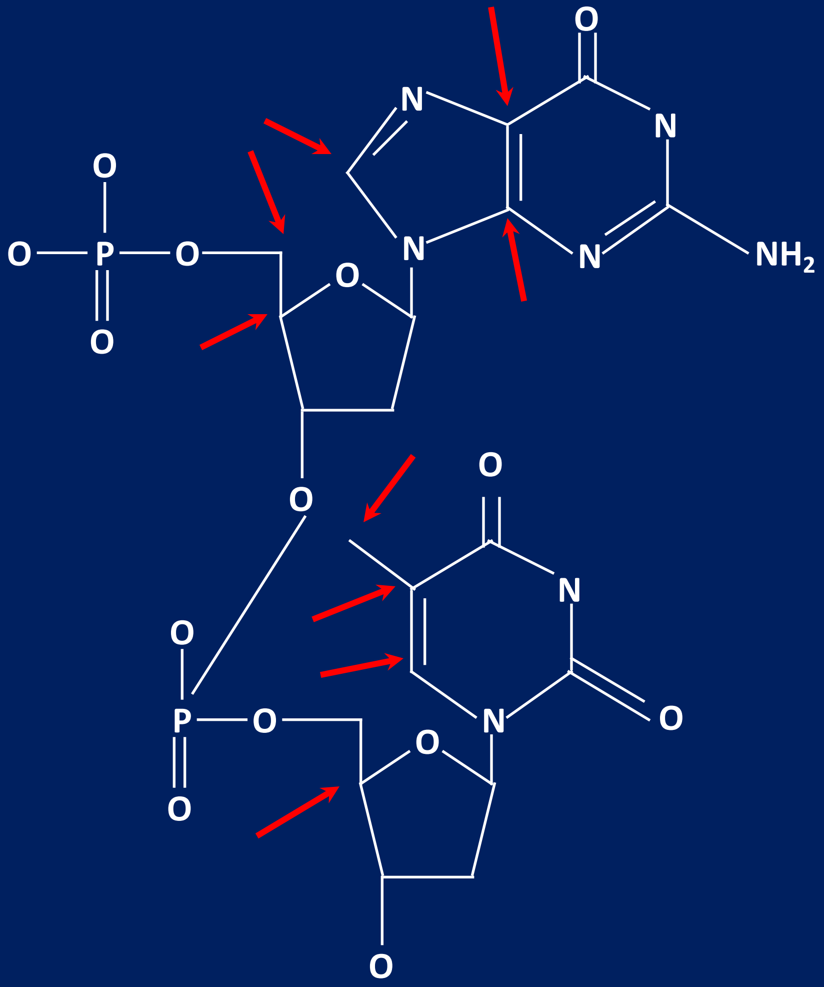
F$_2$-IsoP


O· fatty acid
H_2C
HC—O—C
H_2C
O· $PO_3(CH_2)_2N(CH_3)_3^+$
$O_2^{•-}$ or
R•
OOH
β-cleavage
OH
R
O
trans-4-hydroxyalkenal
Rearrangement
β-cleavage
malondialdehyde
Reduction by cellular peroxidases
OH
Phospholipase A_2
HOOC
8-isoprostane (8-epi-$PGF_{2\alpha}$)

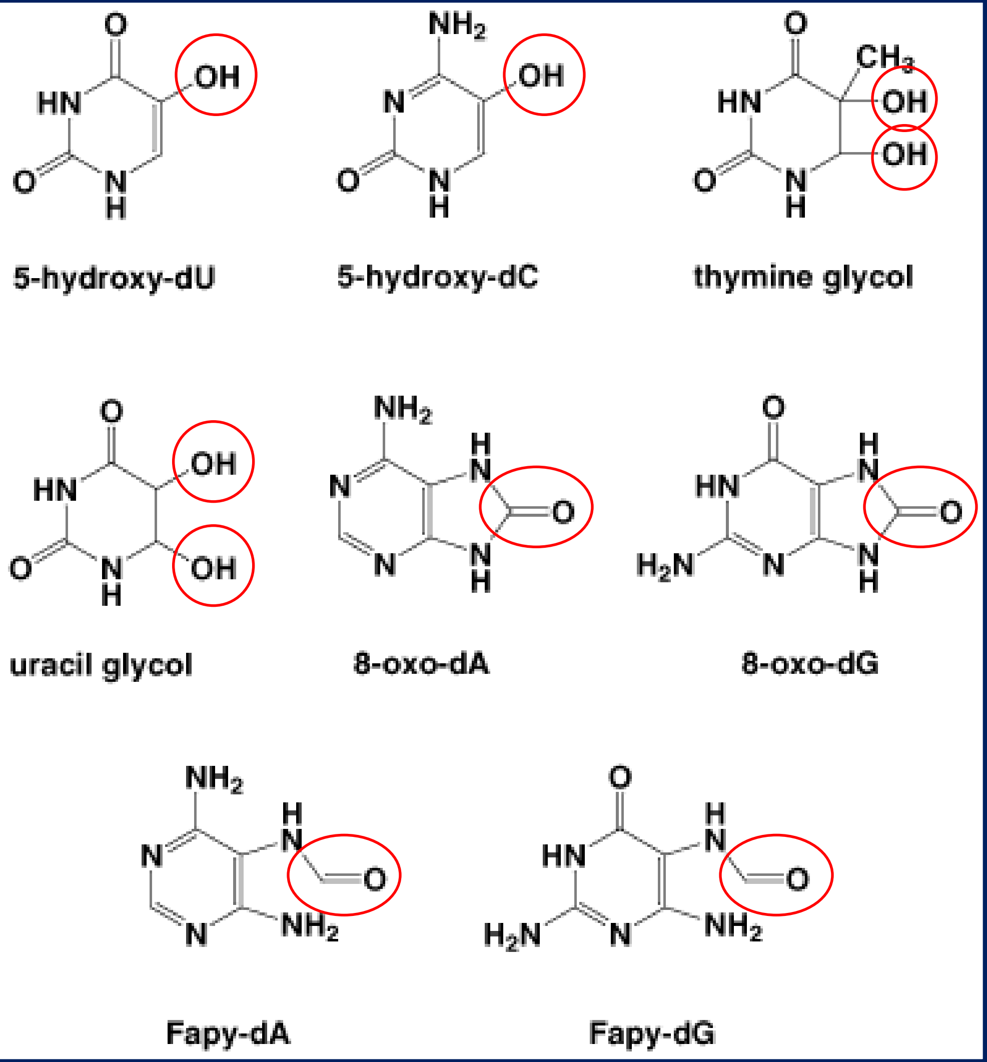
Oxidative DNA Damage

- Correlation with cancers and diseases
- Oxidative DNA lesions by
  - Direct attack
  - Indirect activation of endonuclease enzymes
- Oxidative modification of bases – **mutation**
- Oxidative modification of sugar moieties – **DNA strand break**

Oxidative DNA Damage



Oxidative DNA Damage



- Correlation with cancers and diseases
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  - Direct attack
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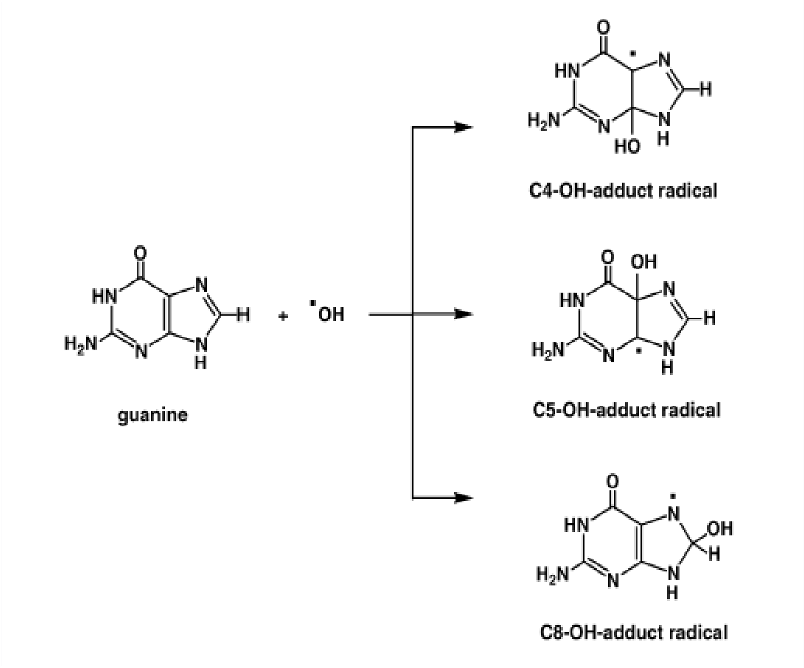
Oxidative DNA Damage



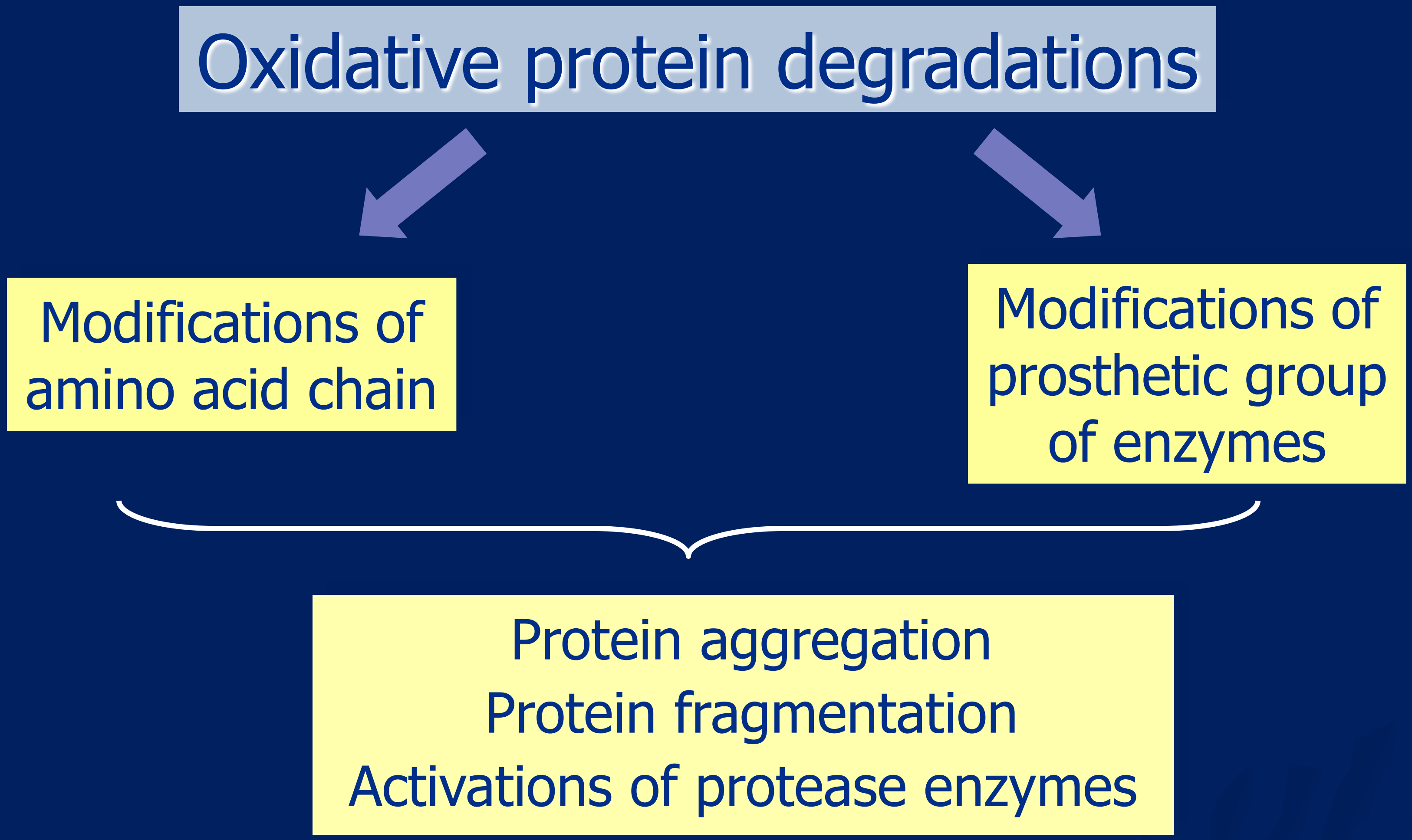
- 8-Hydroxyguanine (8-OH-Gua)
  - GC ⟶ TA transversions (frequently detected in *p53* gene and *ras* protooncogene)
- 2-Hydroxyadenine (2-OH-Ade)
- 8-Hydroxyadenine (8-OH-Ade)
- 5-Hydroxycytosine (5-OH-Cyt)
- 5-Hydroxyuracil (5-OHUra)

- Hydroxylation of guanine residue (dG) to produce 8-OH-dG is the most common biomarker of OH-induced DNA damage
- Detectable in cell, tissues, urine
- Levels can be modulated by antioxidants

Protein Oxidation

- Protein targets
  - Receptors, transport proteins, enzymes, etc
  - Secondary damage – autoimmunity
- Protein oxidation products
  - Protein carbonyl group, 3-nitrotyrosine, other oxidized amino acids
- Most susceptible amino acids
  - Tyrosine, histidine, cysteine, methionine

Protein Oxidation



Oxidative protein degradations

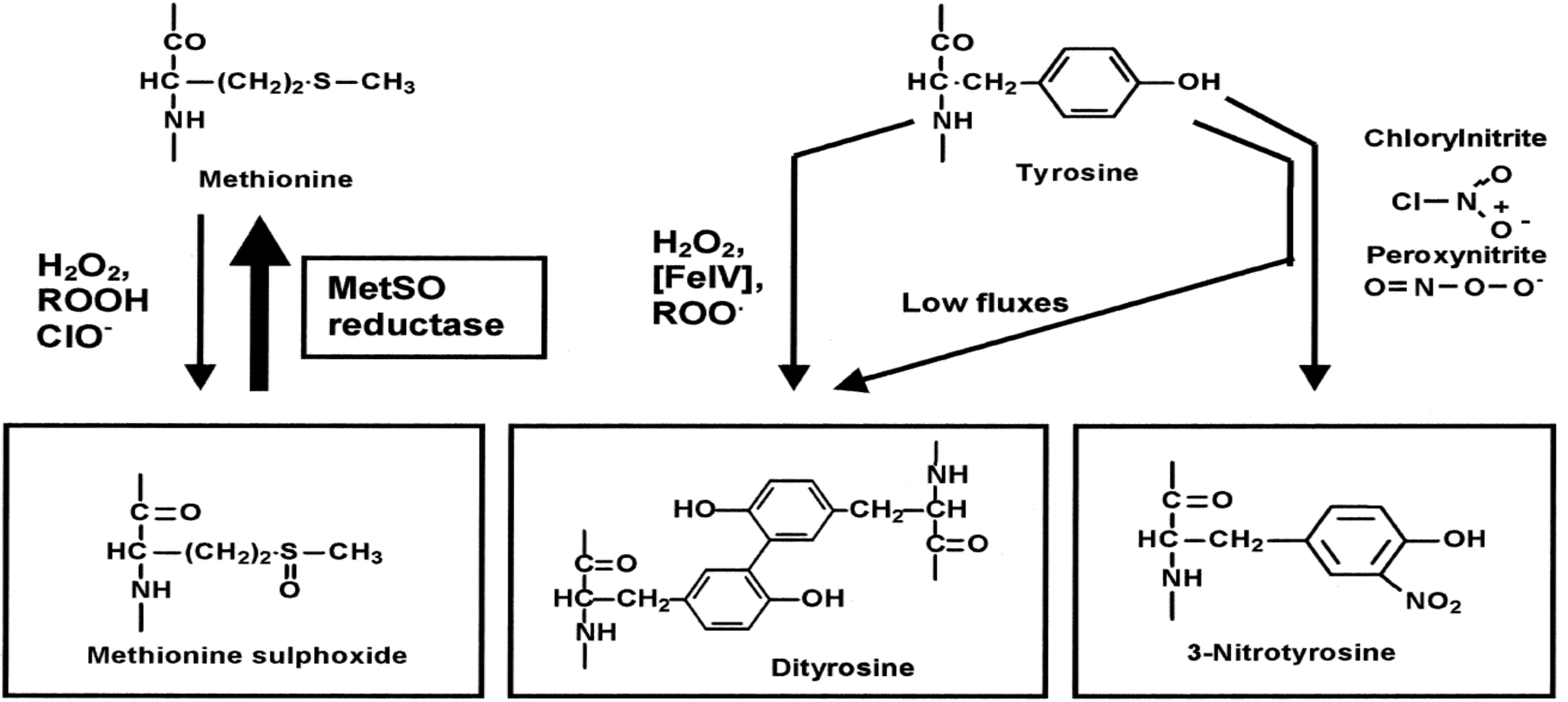
Modifications of amino acid chain

Modifications of prosthetic group of enzymes

Protein aggregation
Protein fragmentation
Activations of protease enzymes

Protein oxidation and nitrosation

(Inflammatory disorders, degenerative disorders, diabetes, lipid peroxidation, ageing)



Methionine

H_2O_2, ROOH ClO^-

MetSO reductase

Tyrosine

H_2O_2, [FeIV], $ROO^\cdot$

Low fluxes

Chlorylnitrite

Peroxynitrite

Methionine sulphoxide

Dityrosine

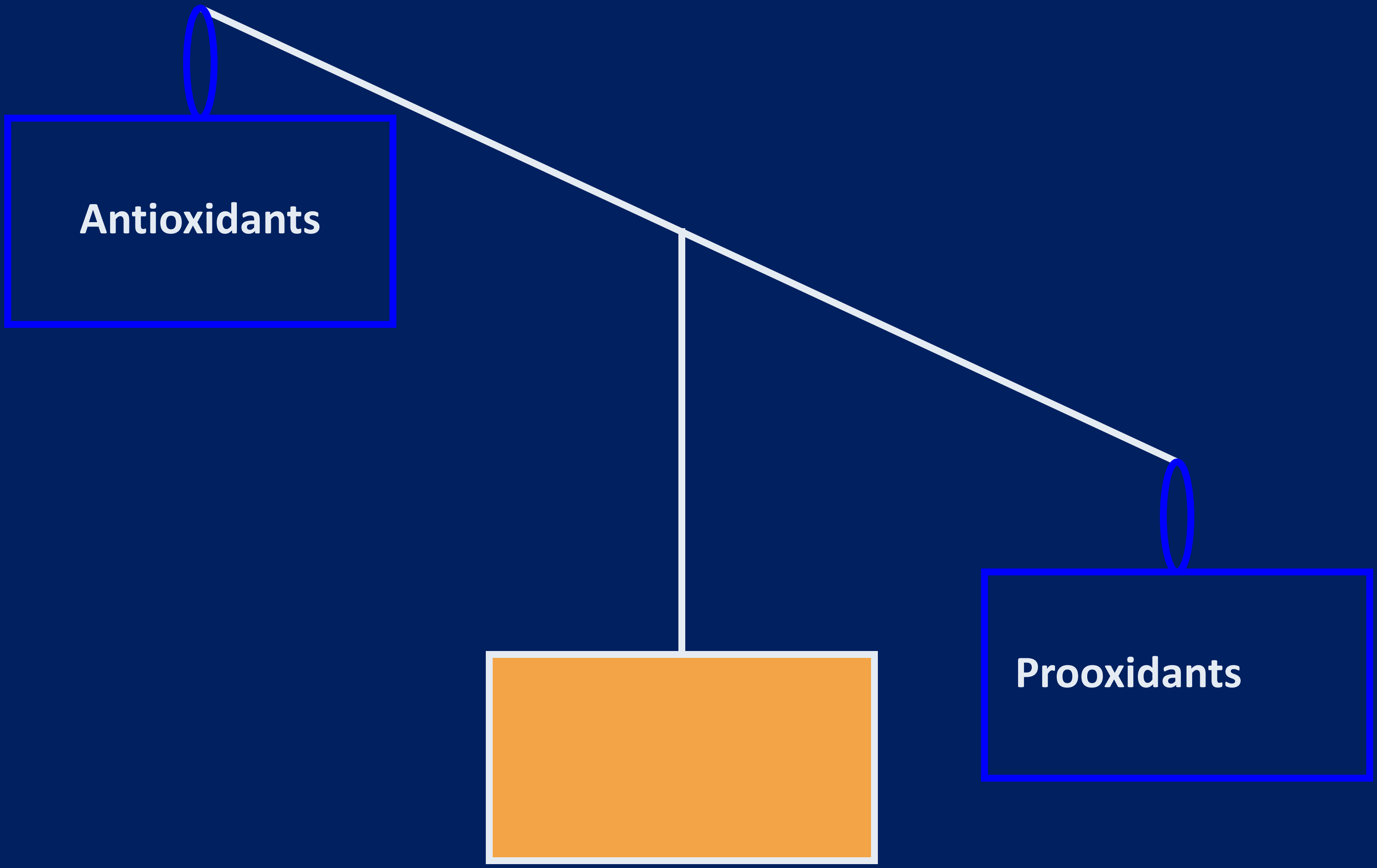
3-Nitrotyrosine

Highly prevalent protein oxidation product

(Haemoglobin is particularly susceptible)

May be increased selectively in vascular dysfunction

Oxidative Stress

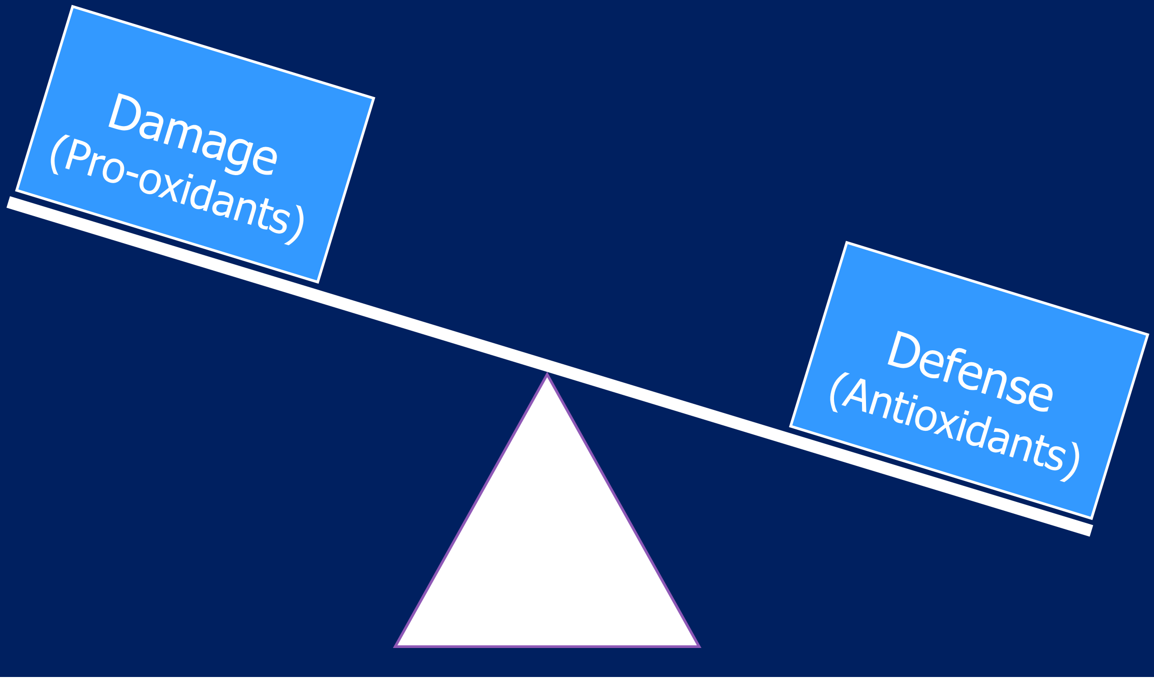


Antioxidants

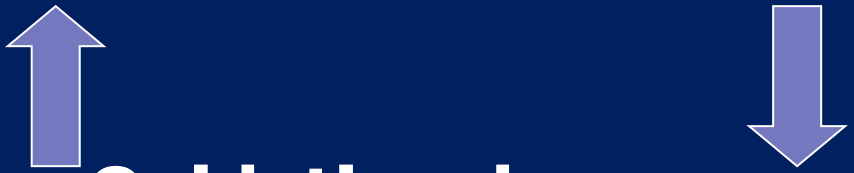
Prooxidants

"An imbalance favoring prooxidants and/or disfavoring antioxidants, potentially leading to damage" -H. Sies

Oxidant-Antioxidant Balance

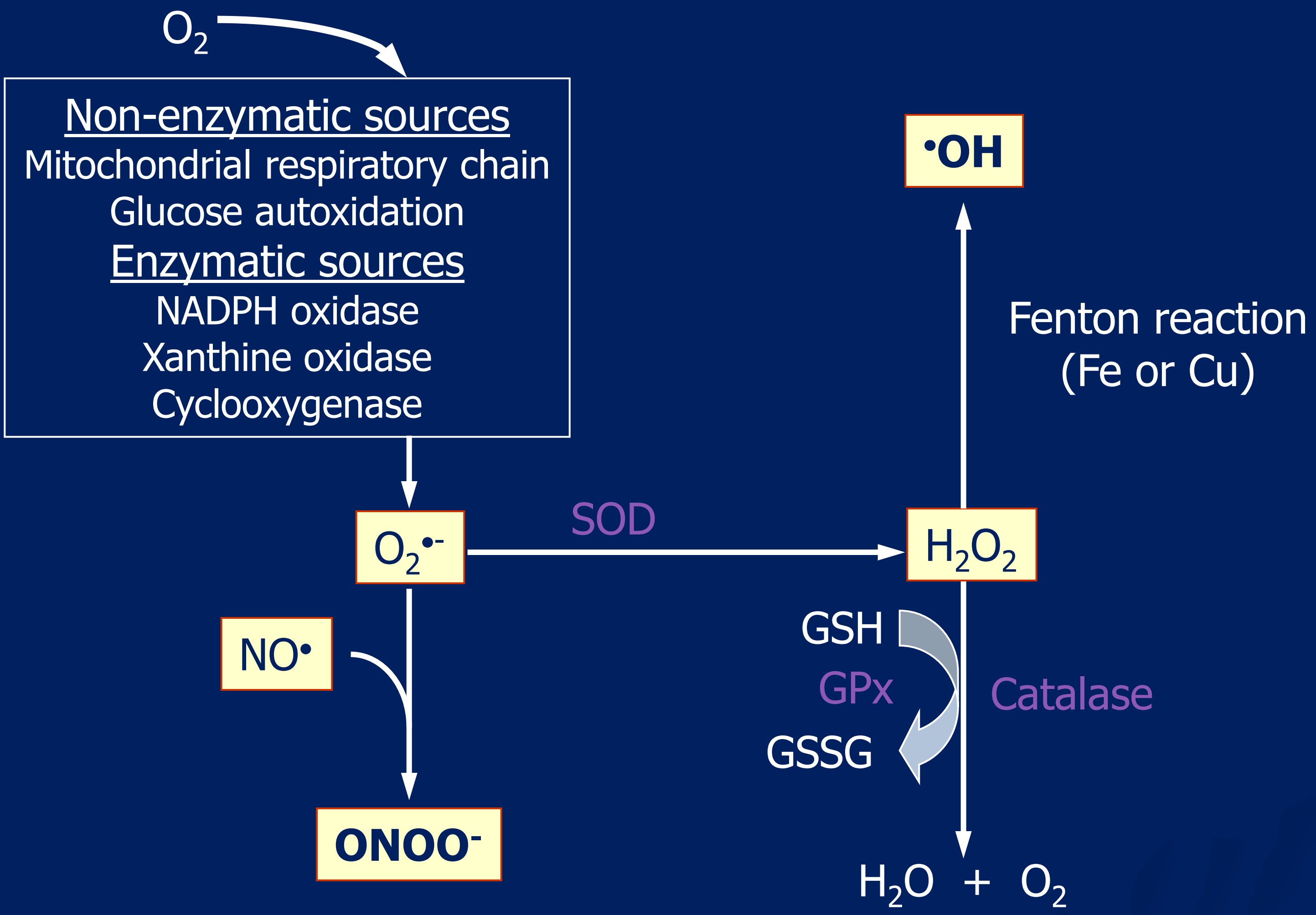


Decrease of antioxidant defense system



Oxidative damage

Oxidative Stress



O_2

Non-enzymatic sources
Mitochondrial respiratory chain
Glucose autoxidation
Enzymatic sources
NADPH oxidase
Xanthine oxidase
Cyclooxygenase

$O_2^{\bullet -}$

SOD

H_2O_2

$^{\bullet}OH$

Fenton reaction
(Fe or Cu)

$NO^{\bullet}$

$ONOO^-$

GSH

GPx

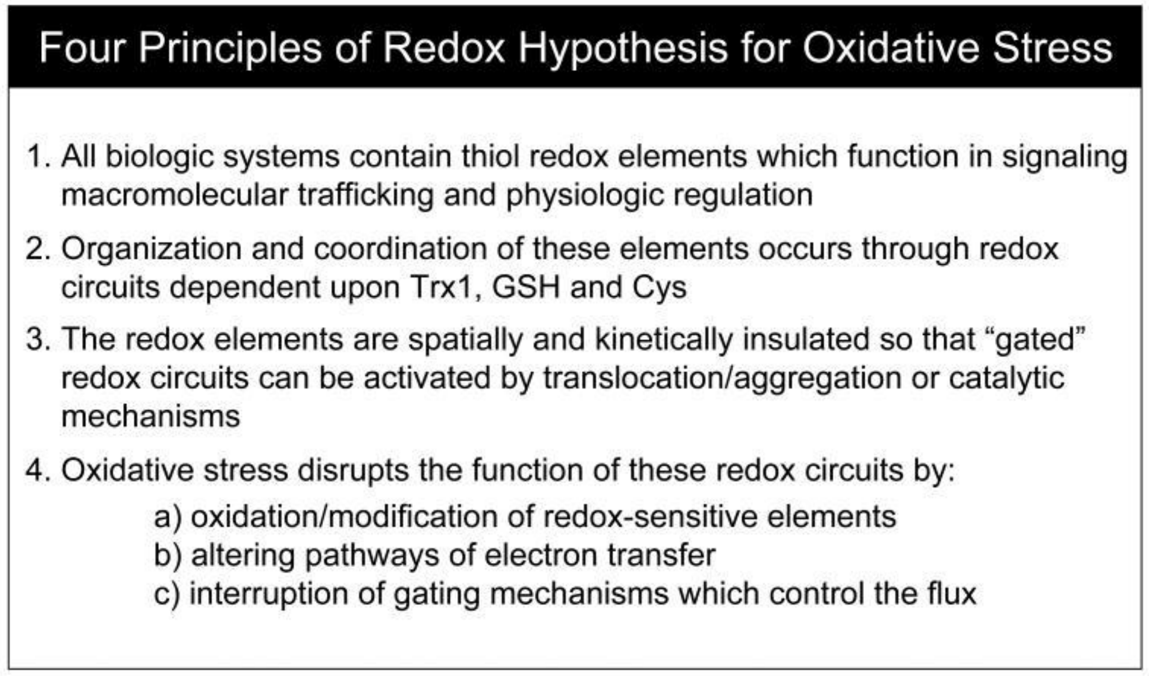
GSSG

Catalase

$H_2O + O_2$

The redox hypothesis for oxidative stress

Diabetes Obes Metab. 2010
October; 12(Suppl 2): 116–125



Four Principles of Redox Hypothesis for Oxidative Stress

1. All biologic systems contain thiol redox elements which function in signaling macromolecular trafficking and physiologic regulation
2. Organization and coordination of these elements occurs through redox circuits dependent upon Trx1, GSH and Cys
3. The redox elements are spatially and kinetically insulated so that “gated” redox circuits can be activated by translocation/aggregation or catalytic mechanisms
4. Oxidative stress disrupts the function of these redox circuits by:
    - a) oxidation/modification of redox-sensitive elements
    - b) altering pathways of electron transfer
    - c) interruption of gating mechanisms which control the flux

Oxidative stress research has largely focused on free radical mechanisms as a contributing factor in disease development but large-scale double-blind interventional trials with free radical scavenging antioxidants have been disappointing in providing little evidence of health benefit.

The redox hypothesis, consisting of four postulates as indicated, was formulated as ***an alternative interpretation of experimental data***

Redefining oxidative stress

Thus, from a mechanistic standpoint, **oxidative stress may be better defined as a disruption of redox signaling and control**.

Adoption of such a definition could redirect research to identify key perturbations of redox signaling and control and lead to new treatments for oxidative stress-related disease processes

Antioxid Redox Signal. 2006 Sep-Oct;8(9-10):1865-79.
Redefining oxidative stress.
Jones DP. http://www.ncbi.nlm.nih.gov/pubmed/16987039

Cellular Defense Mechanisms

- Isolation of generation sites of reactive oxygen species
- Inhibition of propagation phase of reactive oxygen species
- Scavenging of reactive oxygen species
- Repair of the damage caused by reactive oxygen species

Protection Against ROS Damage

- Direct protection against ROS
  - Superoxide dismutase, Glutathione peroxidase, Catalase
- Non-specific reduction system
  - Glutathione, Vitamin C
- Protection against lipid peroxidation
  - Glutathione peroxidase, Vitamin E, β-Carotene, exogenous antioxidants (e.g. flavonoids)
- Sequestration of metals
  - Transferrin, Lactoferrin, Ferritin, Metalothionein
- Repair systems
  - DNA repair enzymes, Macroxyproteinases, Glutathione transferase

Antioxidant Defense System

- **Antioxidant Enzymes**
  - Superoxide dismutase (SOD)
  - Catalase (CAT)
  - Glutathione peroxidase (GPx)
- **Endogenous non-enzymatic antioxidants**
  - GSH, bilirubin
- **Exogenous antioxidant molecules**
  - α-Tocopherol -- prevents oxidation of fatty acids
  - Carotenoids (β-carotene, leutin, lycopene, etc) -- destroy a particularly damaging form of singlet oxygen
  - Ascorbic acid -- radical scavenging, recycling of vitamin E
  - Bioflavonoids -- potent antioxidant activity

Superoxide Dismutase (SOD)

$$2O_2{\bullet}{-} \;+\; 2H^+ \;=\; H_2O_2 \;+\; O_2$$

$$k \sim 2\text{-}4 \times 10^9 \; M^{-1}s^{-1}$$

- Only enzyme known to react with radical
- The presence of SOD implies O2.- produced in cell during normal metabolism
- * SOD is a **primary antioxidant** enzyme

Forms of SOD

Procaryotic SOD

	MW/Da	Subunits
Fe-SOD	40,000	2
Mn-SOD	40,000	2
	80,000	4

Eucaryotic SOD

	MW/Da	Subunits
Mn-SOD	88,000	4
CuZn-SOD	32,000	2
EC (CuZn)	135,000	4
EC Mn-SOD	150,000	2,4

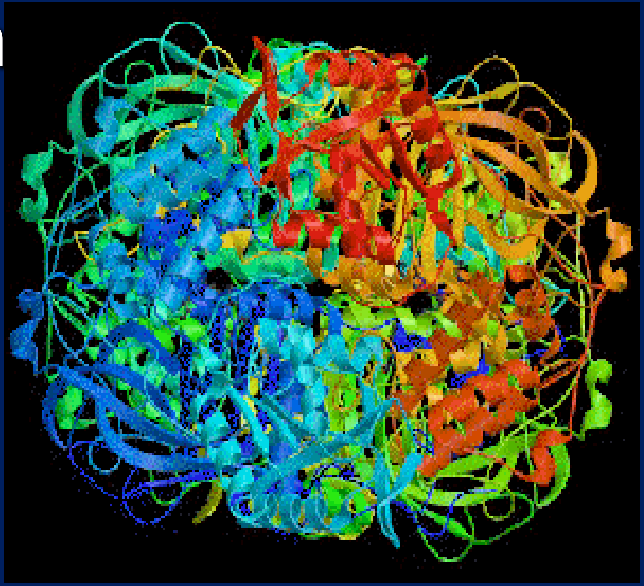
- **CuZn-SOD**
  - Cytoplasm, nucleus, lysosomes
- **Mn-SOD**
  - Mitochondrial matrix
- **EC (CuZn)**
  - Plasma membrane, extracellular
- **EC Mn-SOD**
  - Plasma membrane

Catalase (CAT)

Function : Removes H_2O_2

$$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$$

- Prevents lipid peroxidation and protein oxidation



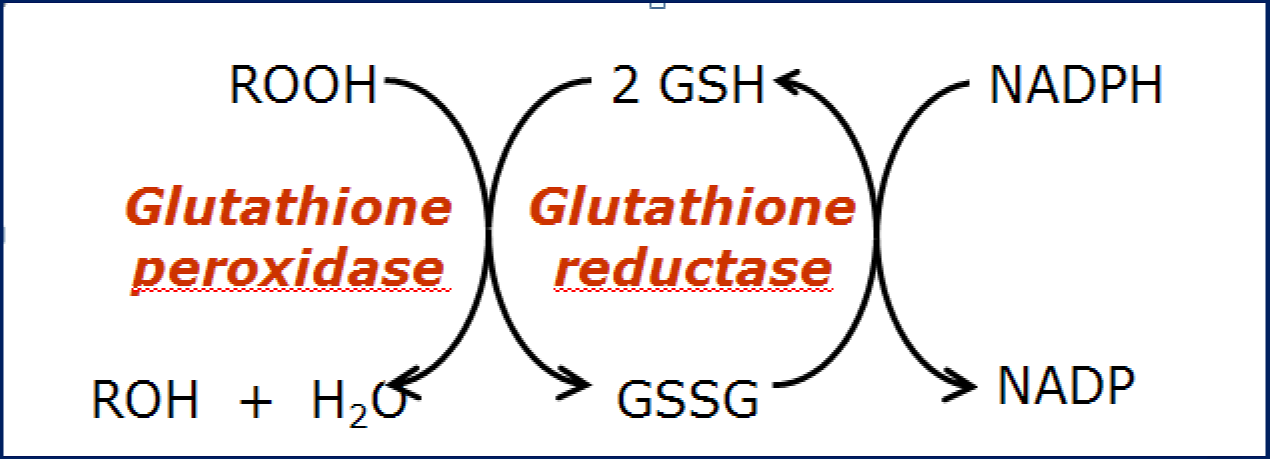
Glutathione Cycle

Glutathione ~ Glu-Cys-Gly

Reduced glutathione (GSH)

Oxidized glutathione (GSSG)

Function : gets rid of H_2O_2 or ROOH (hydroperoxide)



Glutathione Biosynthesis

Two Step-Mechanism

1. By enzyme g-glutamylcysteine synthetase

L-glutamate + L-cysteine + ATP → L-g-glutamylcysteine +ADP + Pi

2. By enzyme glutathione synthetase

L-g-glutamylcysteine + glycine + ATP ⟶ GSH + ADP + Pi

Buthionine sulphoximine (BSO) *inhibits* g-glutamylcysteine synthetase

Cellular GSH ⟶ increase sensitivity to toxicants

Glutathione Peroxidase (GPx)

Function : Removes H_2O_2 & ROOH

$$\mathrm{ROOH} + 2\,\mathrm{GSH} \longrightarrow \mathrm{ROH} + \mathrm{H_2O} + \mathrm{GSSG}$$

Deficiency in GPX leads to oxidative hemolysis

Protects against lipid peroxidation

Selenium

Low Molecular Mass Agents

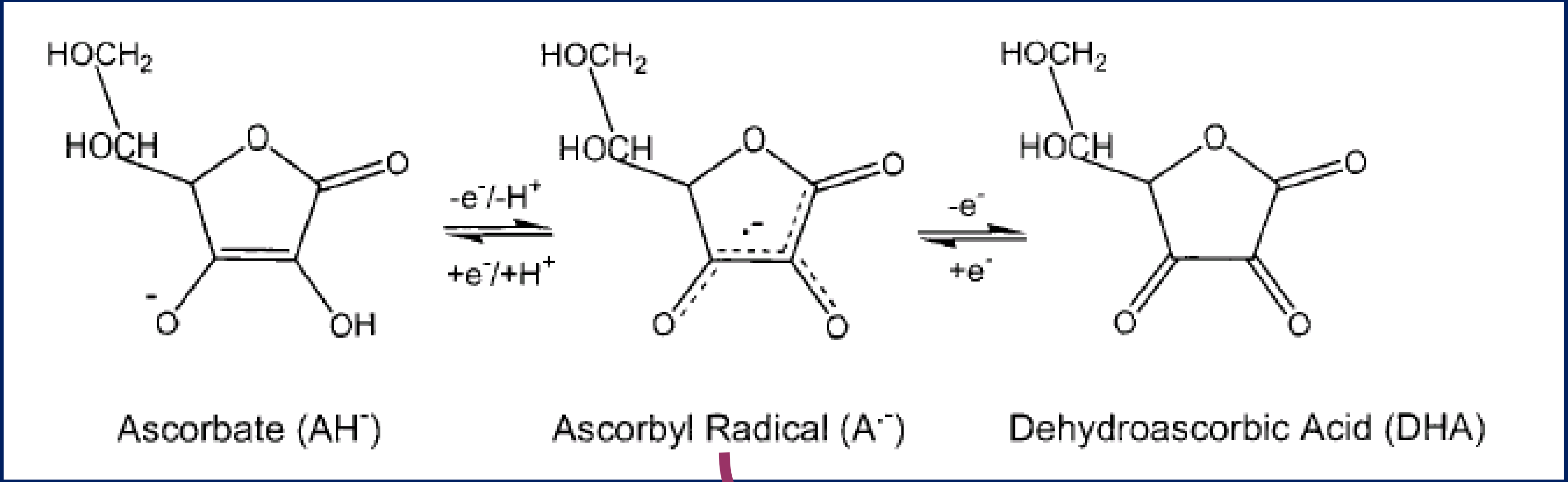
- Compounds synthesized *in vivo*
  - bilirubin, melatonin, lipoic acid, uric acid, etc.

- Compounds derived from the diet
  - Ascorbic acid
  - Vitamin E

Ascorbic Acid

Antioxidant Function

Donate 1 e- → semidehydroascorbate (ascorbyl radical)



Relatively unreactive

Ascorbate-Glutathione Cycle

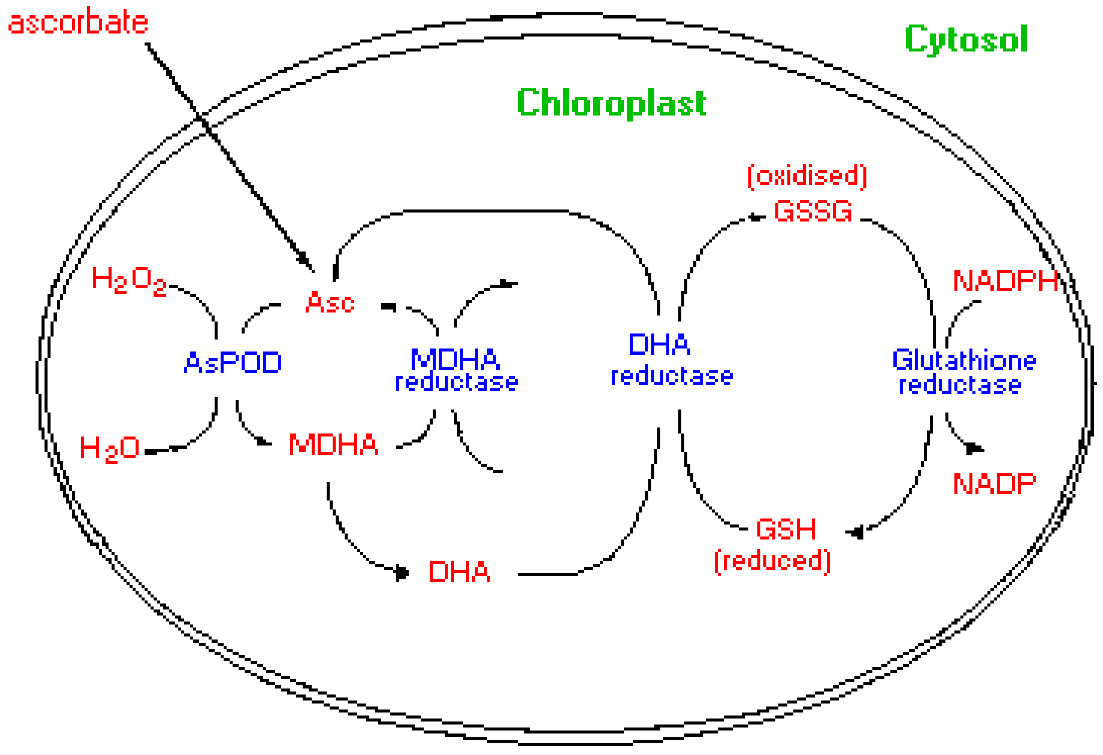


Figure 5. The ascorbate-glutathione cycle. Adapted from Foyer, Lelandais and Kunert, 1994.

$$\text{DHA} + 2\text{GSH} \longrightarrow \text{ascorbate} + \text{GSSG}$$

DHA = dihydroascorbate

Tocopherol

“Chain-breaking antioxidant”

Scavenges peroxy radical

Inhibits chain reaction of lipid peroxidation

Eight naturally-occurring substances

d-α-, *d*-β-, *d*-γ-tocopherols

d-α-, *d*-β-, *d*-γ-tocotrienols

Antioxidant Network

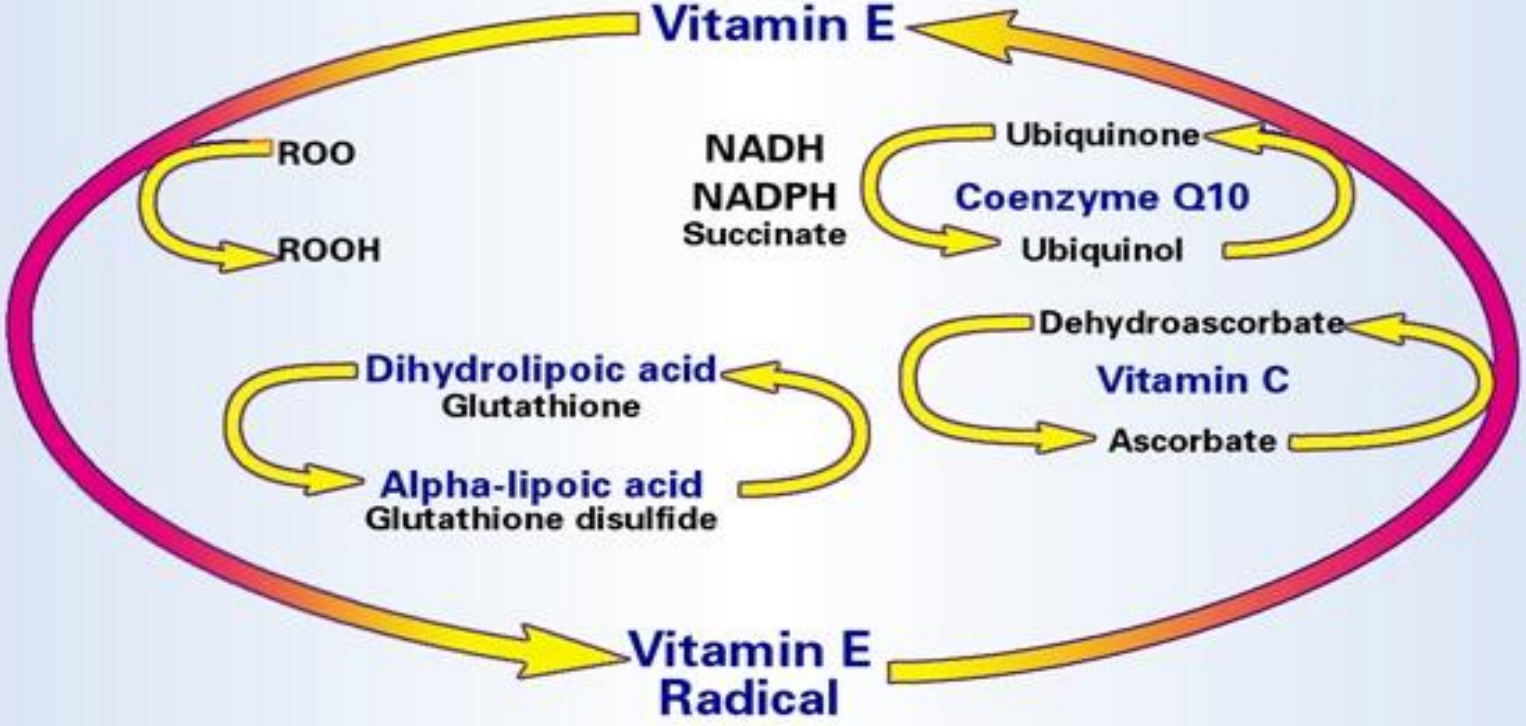
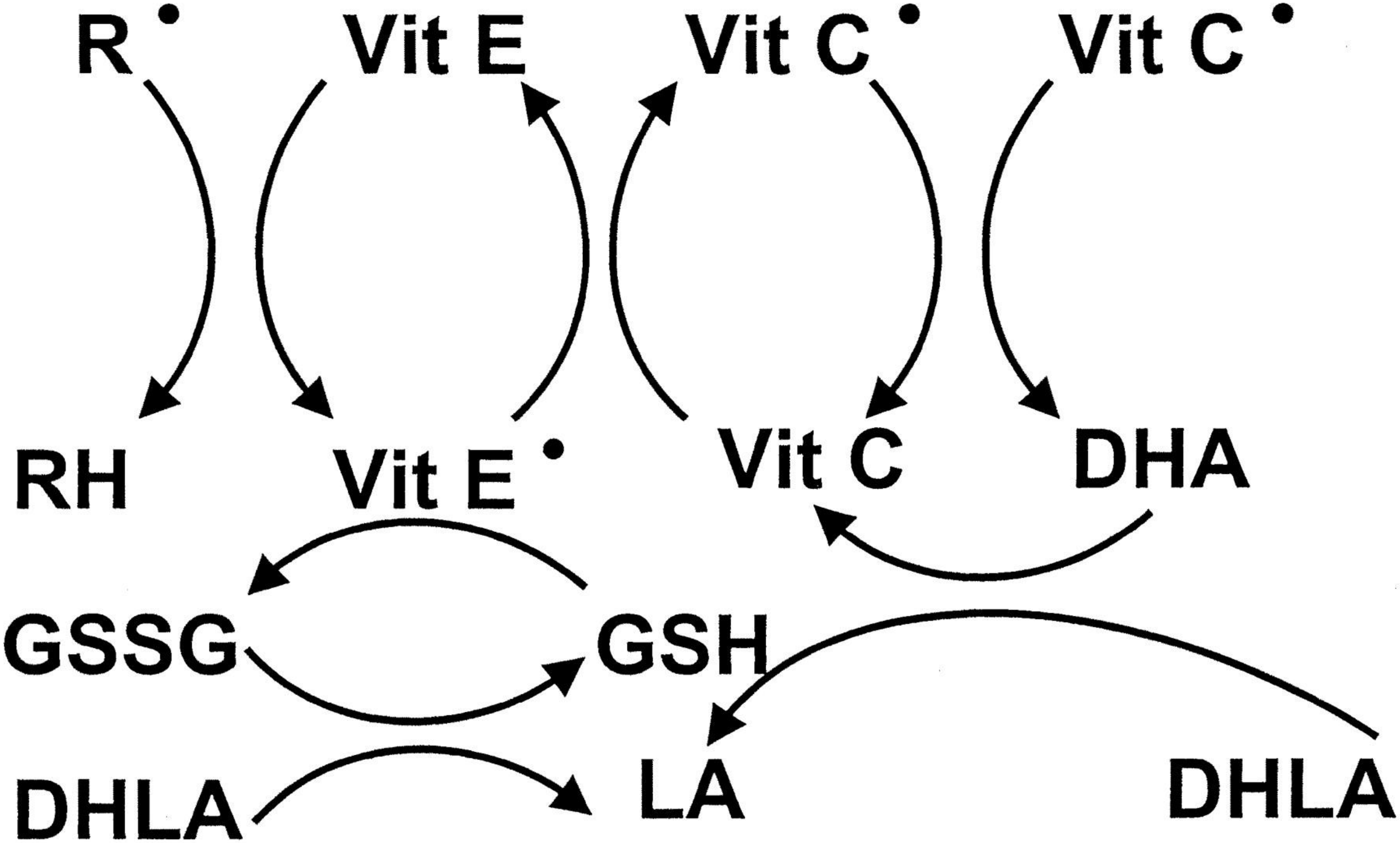


Fig. 1. Antioxidant regeneration pathway. R-lipoic acid recycles vitamins E and C, and in its reduced form, dihydrolipoic acid, acts as powerful antioxidant within mitochondria.

Interaction and regeneration of endogenous antioxidants by LA and DHLA. Interaction and regeneration of endogenous antioxidants occurs through a cooperative set of reactions that can involve many substances (73 74).



R•
Vit E
Vit C•
Vit C•

RH
Vit E•
Vit C
DHA

GSSG
GSH

DHLA
LA
DHLA

Evans J L et al. Endocrine Reviews 2002;23:599-622

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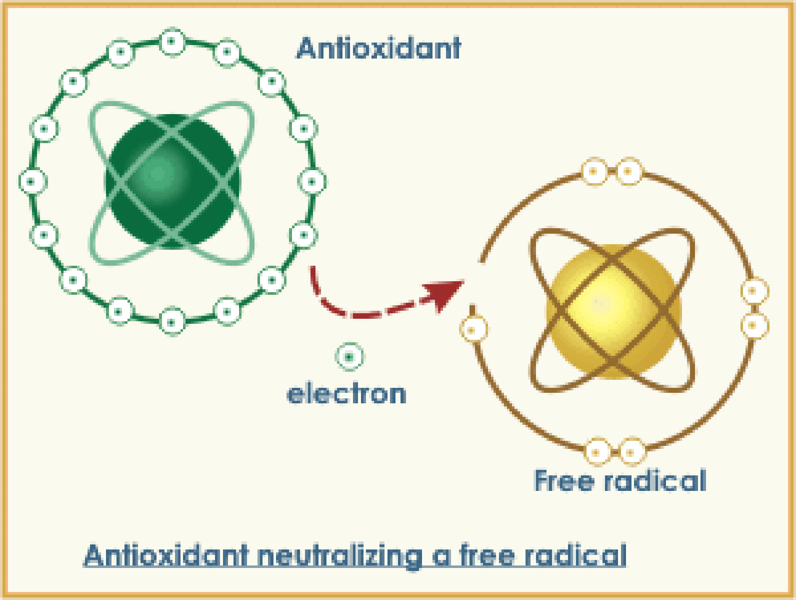
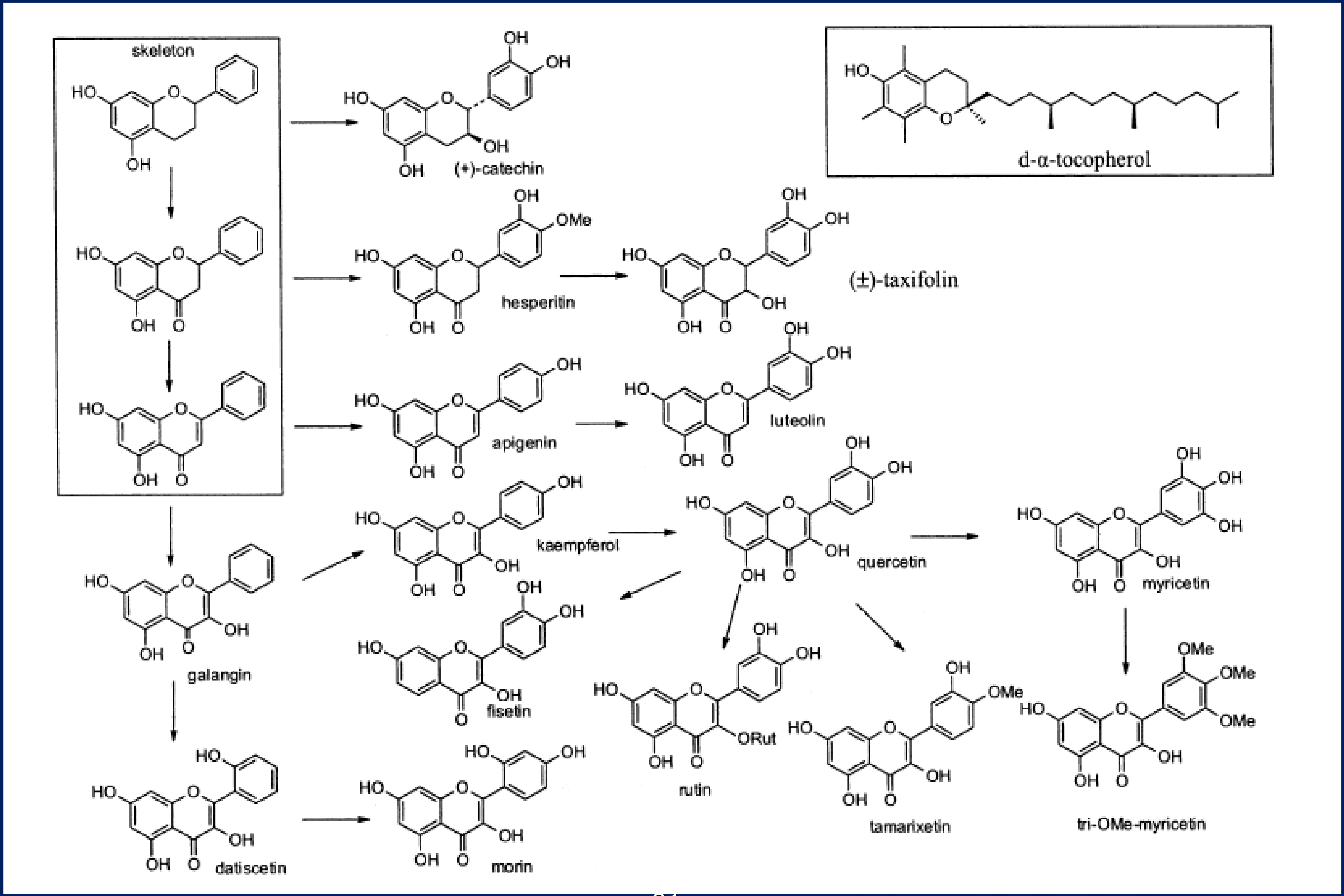
Biological Properties of Natural Antioxidants

- Natural antioxidants
  - Polyphenols (phenolic, flavonoids), carotenoids, lycopene, etc
- Electron donor property
  - Ability of antioxidant to donate an electron to a species (free radical) – reducing property
  - Antioxidant remains stable



Antioxidant neutralizing a free radical

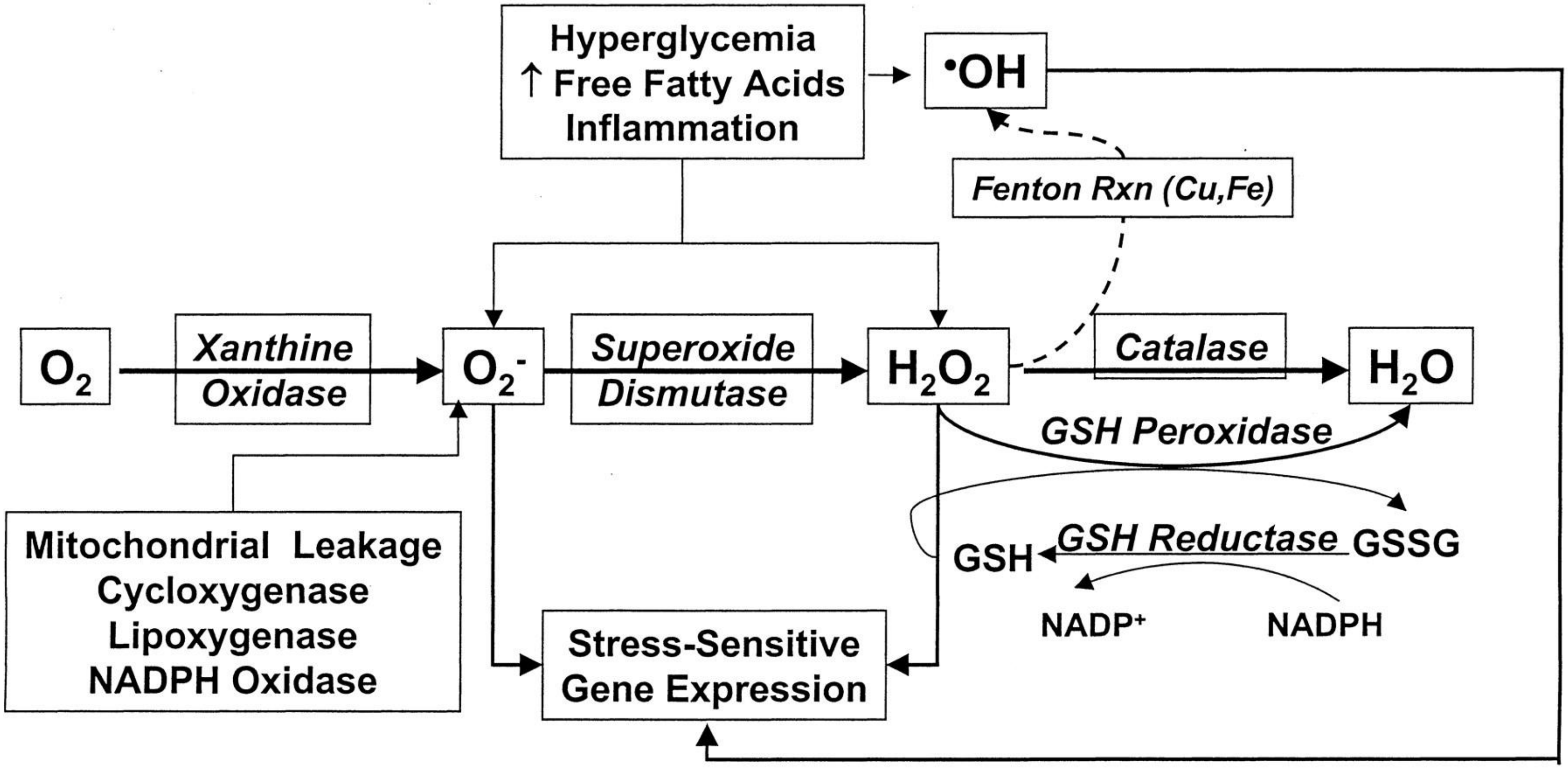
Basic Ring System of Flavonoids



Roles of Free Radicals in Biological Systems

- Enzyme-catalyzed reactions
- Electron transport in mitochondria
- *Signal transduction & gene expression*
- *Activation of nuclear transcription factors*
- Oxidative damages of molecules, cells, tissues
- Antimicrobial actions
- Aging & diseases

Exogenous and endogenous stimuli leading to ROS generation and activation of stress-sensitive gene expression.



Evans J L et al. Endocrine Reviews 2002;23:599-622

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